

Structural Biomaterials

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Structural Biomaterials

THIRD EDITION

• *JULIAN VINCENT* •

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• P R E F A C E •

It's been twenty years since this book was last revised, by someone who resembled me in many respects. In the present edition I have tried to retain that author's challenge and enthusiasm tempered (I hope) by knowledge and hard-edged simplicity.

Those twenty years have seen major advances that impinge on the study of structural biomaterials. Knowledge of the molecular structure and properties of biological polymers has expanded, initially under the guise of molecular biochemistry, which developed into "structural biology." Nanotechnology, in which materials scientists have burrowed down from millimeters to micrometers to nanometers, is meeting biologists (as many biochemists like to call themselves) coming up. This marriage is starting to produce a new class of materials, including biomimetic and "intelligent" ones, as the roots of the complexity and adaptiveness of biological materials become better known. This understanding will inevitably feed back to biology.

New models, such as cellular materials, are being developed for complex materials (or are they structures?), which, in turn, further the understanding of the mechanics of plants. The principles of biological design—function, shape, and structure—are being applied to architecture. Biomechanics is increasingly being used by paleontologists to expand their understanding of the lifestyle of extinct animals and plants. They can calculate the physical limits of performance implied by a particular morphology, and hence its behavioral possibilities.

This wide-ranging use of the study of engineering design of biological materials and structures is a product of the many disciplines it encompasses. Generally, no single person has the knowledge and intuition to appreciate all the aspects of a particular problem. In a viable research group, engineering, materials science, polymer physics, mathematics, zoology, physical chemistry, ecology, and botany may all be more or less equally represented and equally important. Such a diversity of approach is stimulating to all those involved. And just as biomechanics cuts across wide boundaries of science, so it cuts across phylogenetic boundaries within biology. Biomechanics is about functions and mechanisms. Thus the ideas involved in studying fracture or composite materials or plasticizers or ceramics can be applied equally to animals and plants, since all organisms are limited to a relatively small range of materials and structures. They are also subject to similar ranges of forces within the environment, dependent on such generalities as size. Different phyla will be found to have come to terms with these forces in different ways, but since they are all subject to the same mechanical and engineering limitations (which is why pigs can't fly), the general principles remain the same. Thus biomechanics combines with comparative functional morphology and becomes the refinement and redefinition of one of the most traditional approaches to biology. Biomechanics is neomorphology. It is morphology plus numbers and so is "hard" science. And in the stressful environment,

mechanical functions and properties are as important as the developmental and physiological functions that generate the structures and materials. A subject area that, to the traditionalist, is so diverse cries out for a novel form of categorization. The last chapter includes an account of the development of biomimetics, biology's challenge to engineering. Is technology the best way to run the Earth, or can biology (and biologists) teach us how better to live and survive?

In this book I have combined several approaches but tried throughout to maintain a viewpoint that the biologist (and I am first and foremost a lover of animals and plants) can appreciate and understand. It seems necessary to provide a framework of theory, so the first chapter explains and expounds some of the basic concepts, but it is as much an appendix as an introduction. There follow five chapters in which the molecular rather than the mathematical approach is developed, from proteins and polysaccharides through ceramics. One of the outcomes of this approach is explored in a chapter on biomimetic materials and comparisons between biology and the ideas of materials science and engineering that have been used to describe it.

I have, unashamedly, included only those ideas and topics I find interesting and feel I can understand. I apologize profoundly to those whose work I appear to find, by implication of omission, boring or obscure! Thus this book is a statement, as well as a product, of my enthusiasm. It is therefore bound to be bitty and unbalanced but with odd thoughts and asides that I hope will stimulate and perhaps even amuse.

My thanks are due to many friends and colleagues, some of them past students. I was going to list them, but their names all appear somewhere in this volume. Even so, I must particularly mention the late Jim Gordon, who was the first person to make this all seem possible for me.

Structural Biomaterials

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Basic Elasticity and Viscoelasticity

In the physically stressful environment there are three ways in which a material can respond to external forces. It can add the load directly onto the forces that hold the constituent atoms or molecules together, as occurs in simple crystalline (including polymeric crystalline) and ceramic materials—such materials are typically very rigid; or it can feed the energy into large changes in shape (the main mechanism in noncrystalline polymers) and flow away from the force to deform either semipermanently (as with viscoelastic materials) or permanently (as with plastic materials).

1.1 Hookean Materials and Short-Range Forces

The first class of materials is exemplified among biological materials by bone and shell (chapter 6), by the cellulose of plant cell walls (chapter 3), by the cell walls of diatoms, by the crystalline parts of a silk thread (chapter 2), and by the chitin of arthropod skeletons (chapter 5). All these materials have a well-ordered and tightly bonded structure and so broadly fall into the same class of material as metals and glasses. What happens when such materials are loaded, as when a muscle pulls on a bone, or when a shark crunches its way through its victim's leg?

In a material at equilibrium, in the unloaded state, the distance between adjacent atoms is 0.1 to 0.2 nm. At this interatomic distance the forces of repulsion between two adjacent atoms balance the forces of attraction. When the material is stretched or compressed the atoms are forced out of their equilibrium positions and are either parted or brought together until the forces generated between them, either of attraction or repulsion, respectively, balance the external force (figure 1.1). Note that the line is nearly straight for a fair distance on either side of the origin and that it eventually curves on the compression side (the repulsion forces obey an inverse square law) and on the extension side. With most stiff materials the extension or compression is limited by other factors (see section 1.6) to less than 10% of the bond length, frequently less, so that the relationship between force and distance is essentially linear. When the load is removed, the interatomic forces restore the atoms to their original equilibrium positions.

It is a fairly simple exercise to extend this relationship to a material such as a crystal of hydroxyapatite in a bone. This crystal consists of a large number of atoms held together by bonds. The behavior of the entire crystal in response to the force is the summed responses of the individual bonds. Thus one arrives at the phenomenon described by Hooke as *ut tensio, sic vis*, “as the extension, so the force.” In other words,

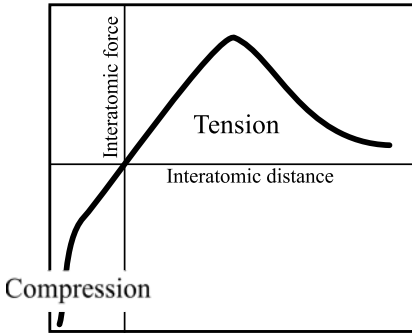


Figure 1.1. Stress–strain curve at the atomic level for a “perfect” material. The origin represents the equilibrium interatomic distance. On either side of the origin the curve is nearly straight.

extension and force are directly and simply proportional to each other, and this relationship is a direct outcome of the behavior of the interatomic bond. However, when one is dealing with a piece of material it is obvious that measurements cannot conveniently be made of the interatomic distance (though they have been made using X-ray diffraction, which confirms the following). What is actually measured is the increase in length of the whole sample or a part of the sample (making the verifiable assumption that in a homogeneous material one part will deform as much as the next). This difference is then expressed as a function of the starting length called the *strain*, ϵ . Strain can be expressed in a number of ways, each offering certain advantages and insights into the processes of deformation. The most commonly encountered form is conventional, nominal, engineering, or Cauchy strain, which is the increase in length per unit starting length:

$$\epsilon_c = \frac{\Delta l}{L_0}. \quad [\text{Eq. 1.1}]$$

This estimate of extension works well if the material is extended by no more than a tenth of its starting length. Strain is expressed either (as in this text) as a number (e.g., 0.005) or as a percentage (e.g., 0.5%).

The force acting on each bond is a function of the number of bonds available to share the load. Thus if the area over which the force acts is doubled, then the load carried by each bond will be halved. It is therefore important, if one is to bring the data to the (notionally) irreducible level of the atomic bond, to express the force as a function of the number of bonds that are responding to it. In practice this means expressing the force as force divided by the area across which the force is acting, which is called the *stress*, σ :

$$\sigma = \frac{f}{A_0}. \quad [\text{Eq. 1.2}]$$

However, just as with strain, this simple equation is suitable only for small extensions.

In SI units, the force is expressed in newtons (a function of mass and the acceleration due to gravity: one newton is approximately the force due to 100 g, which can be produced by an average apple falling under the influence of gravity), the area in

square meters. One newton acting over an area of one square meter is a pascal (Pa). Other units are in use in many parts of the world. For instance, in the United States the unit of force is the dyne (the force exerted by one gram under the influence of gravity), and the unit of area is the square centimeter. One dyne per square centimeter is one hundred-thousandth (10^{-5}) of a pascal. Traditional engineers in Britain often use pounds and square inches as their measures of “force” and area.

The slope of the straight, or Hookean, part of the curve in figure 1.1 is characteristic of the bond type and is a function of the energy of the bond. For the same reason, the ratio of stress to strain is a characteristic of a material. This ratio is the stiffness or Young’s modulus, E :

$$E = \frac{\sigma}{\epsilon}. \quad [\text{Eq. 1.3}]$$

The units of E are the same as for stress, since strain is a pure number. Graphs showing the relationship between stress and strain are conveniently plotted with the strain axis horizontal and the stress axis vertical, irrespective of whether the relationship was determined by stretching the test piece in a machine and recording the developed forces or by hanging masses onto the test piece and recording the extension. Do not be surprised if it takes a long time for the mental distinctions between stress and strain to become totally clear. Not only are the concepts surprisingly difficult to disentangle, but the confusion is compounded by their uncritical use in everyday speech.

One other characteristic of Hookean materials is that they are elastic. That is to say, they can be deformed (within limits) and will return to their original shape almost immediately after the force is removed (*almost* immediately because the stress wave travels through the material at the speed of sound in that material. Thus when you pull on the brake lever of your bicycle, the brake blocks begin to move a short time later, the time dependent partly on the speed of sound in the steel cable and partly on the length of the cable). This use of the word *elastic* must not be confused with the use of the term as in “elastic band,” where “elastic” is taken to mean highly extensible.

Young’s modulus is a measure of stiffness in simple extension or compression. There are ways of deforming a material that have different effects on the interatomic forces and therefore different effects on the material. Such a mode of deformation, frequently met, is shear. (Another mode of deformation—volume change, from which is derived the bulk modulus—is ignored here.) As with Young’s modulus, the shear modulus is defined as the ratio of stress to strain. The shear stress, τ , is defined as (figure 1.2)

$$\tau = \frac{f}{A_s}. \quad [\text{Eq. 1.4}]$$

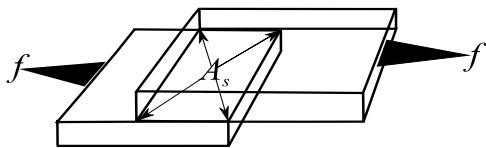


Figure 1.2. Conditions for the definition of one-dimensional shear stress (Eq. 1.4).

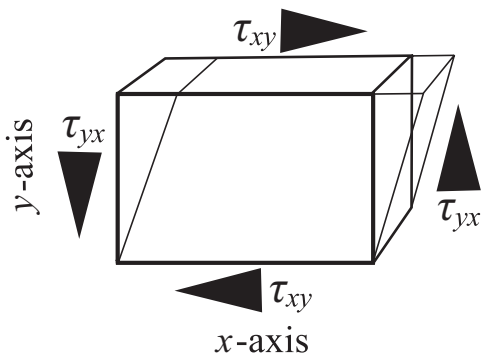


Figure 1.3. Conditions for the definition of two-dimensional shear stress (Eq. 1.5).

The shear strain is defined somewhat differently (figure 1.3). The strain, y , is measured in radians, and the shear modulus, G , is given by

$$G = \frac{\tau}{y}. \quad [\text{Eq. 1.5}]$$

The simple picture given here is for isotropic materials whose structure and, therefore, mechanical response, is the same in all directions. Young's modulus and the shear modulus in an isotropic material can be related to each other by the expression

$$G = \frac{E}{2(1 + \nu)}, \quad [\text{Eq. 1.6}]$$

where ν is Poisson's ratio. This important ratio is discussed at greater length in section 4.3. A material that is Hookean in extension is usually Hookean in shear. The mathematics for high strain shear deformation is not considered here and, indeed, remains to be established!

1.2 Non-Hookean Materials and High Strains

With greater deformation, another form of strain—true or Hencky strain—is a better indicator of what is going on in the material. With true strain, each small extension is expressed as a fraction of the immediately preceding or instantaneous length. It is slightly more cumbersome to calculate,

$$\epsilon_H = \ln\left(\frac{L_0 + \Delta l}{L_0}\right) = \ln(1 + \epsilon_C), \quad [\text{Eq. 1.1a}]$$

and has the curious property that the sample does not “remember” its strain history. True strain is an instantaneous measure of strain. Figure 1.4 compares true and conventional strain, showing that the mutual deviation is far greater in compression.

At larger strains (greater than 0.1 or so), Poisson's ratio effects in an isotropic material (section 4.3) will cause the sample to become narrower, reducing the area over which the force is being transmitted. This will cause the true stress to increase at a

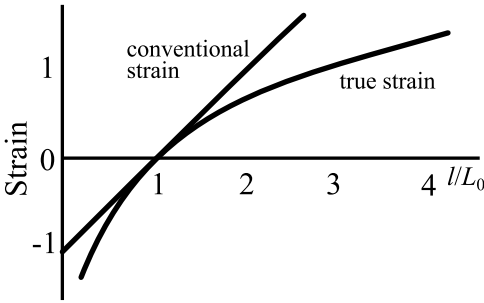


Figure 1.4. Comparison of true and conventional (“engineering”) strain plotted against extension ratio.

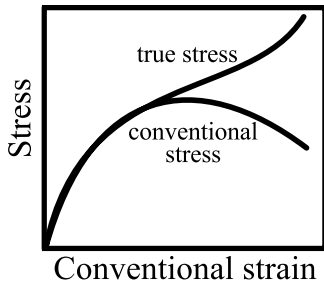


Figure 1.5. Comparison of true and conventional (“engineering”) stress plotted against conventional (“engineering”) strain.

higher rate than the conventional stress (figure 1.5). However, since, as will be seen, Poisson’s ratio frequently varies with strain, especially with soft biological materials that are complex, extensible, and fibrous, it is not possible to give a universal formula for calculating true stress from the starting conditions. The cross-sectional area has to be measured at the particular strain for which the stress is to be calculated. To give you a feel for the relationship between engineering stress and true stress, assume that Poisson’s ratio varies in the same way as a rubber, that is to say, the volume of the material remains constant (for many biological materials a doubtful assumption). Thus if the cross-sectional area at any time is A , and A_0 the area at zero strain (L_0), then

$$A(L_0 + \Delta l) = A_0 L_0, \quad [\text{Eq. 1.2a}]$$

so

$$A = \frac{A_0 L_0}{(L_0 + \Delta l)} = \frac{A_0}{(1 + \epsilon_c)},$$

so that

$$\sigma_H = \frac{f}{A} = \frac{(1 + \epsilon_c)f}{A_0} = (1 + \epsilon_c)\sigma_c,$$

which relates the true stress to the apparent or engineering stress.

Both true and conventional methods of expressing stress and strain are used; in the small-strain elastic range, the conventional measures are more usually used and are more convenient, though not strictly accurate. However, at the strains that soft

biological materials reach, true stress and strain are the proper indicators of what is happening in the material, although these parameters are seldom used. When the material starts to yield (section 1.6), even true stress and strain are inadequate, since neither is uniform across the yield zone. Even so, it seems reasonable to use these measures rather than the conventional parameters, although finite element modeling is probably the preferred compromise. However, since all biological materials show some form of relaxation (section 1.4), an estimate of cross-sectional area for the calculation of true stress has to be made instantaneously. In practice, where such data are required, it is often found that the best technique is to record the test with a number of cameras using split-screen video and to make the necessary measurements of the specimen after the test is completed. This sort of complexity at the practical level goes a long way to explaining why there are so few data on biological materials in which true stress has been measured. When it is measured it is often found to be distributed nonuniformly, so that the assumption of affine (i.e., average or distributed) deformation is not valid.

1.3 The Energy Approach

It is often easier to consider elasticity not as stress and strain but as their product—that is to say, energy. When material is deformed (stretched, perhaps), energy (usually referred to as *strain energy*) is stored in the deformation of its bonds, and it is this energy that brings the material back to its original shape—or perhaps not, since that energy can be dissipated in a number of ways, such as heat, sound, surface energy, plastic deformation, or kinetic energy. With a Hookean material the strains are relatively small, and all the energy is stored in stretching the interatomic bonds, termed the *internal energy*. However, if the material is made of relatively long and unrestrained molecules, the energy can also be stored in changes in their shape and mobility, termed the *entropic energy*. This is typical of the long-range elasticity exhibited by rubbers, which can stretch up to six times their original length. When a rubbery material is deformed, its molecules lose mobility, and the energy that has been powering their random movements is dissipated as heat. If you stretch a rubber band while you hold it to your lip (very sensitive skin), you will detect an increase in temperature. Relax the band, and the molecules resume their motions, taking energy from their surroundings, and you will feel the band go cool. The entropic component can be characterized by this exchange of heat. In simple terms,

$$A = U - TS, \quad [\text{Eq. 1.7}]$$

where A is the Helmholtz free energy, U is the internal energy component, and $-TS$ is the entropic component, made up of temperature, T , and entropy, S . Note that the entropic component is negative. If you increase the temperature of a material that relies on internal energy for its elastic behavior, it will expand, but an entropic-based material will contract. This is the corollary of the experiment with the rubber band.

To introduce external work (i.e., your stretching the material) we have to introduce force, f , multiplied by change in length, dl :

$$dA = -PdV - SdT + fdl. \quad [\text{Eq. 1.8}]$$

This formulation can now be developed to give the basis for measurement of the mechanical properties of a material at a variety of temperatures, which yields the relative contributions of the internal and entropic components of the elastic restoring force. But we need to see what this measurement means in terms of molecular interactions, since this is the starting point for biology. How does the entropic component work at the molecular level?

A technical rubber is composed of very long chains (molecular weight of about 10^5) of one or more monomer units, with each unit more or less freely jointed into the chain, so that each joint allows a wide range of movement. This motion is called “free rotation” about the bonds of the backbone and is what distinguishes a rubbery polymer from a crystalline one: in a crystalline polymer (or in areas of crystallinity) the units cannot move freely because they are packed so closely, and rubbery behavior is impossible. In fact, it takes more than one monomer unit or residue to make a freely rotating unit or “random link,” because the monomer units are of a finite size and shape and so cannot move with absolute freedom without hitting their neighbors (“steric hindrance”). With paraffin chains with a tetrahedral valence angle it takes three C — C links to make up a freely rotating or equivalent random link; with *cis*-polyisoprene units, as in rubber made from the latex of *Hevea brasiliensis*, the number of monomer units per random link is 0.77, since there are four bonds to each isoprene unit (Treloar 1975). Under the influence of Brownian motion the free rotation of the equivalent random links about the backbone of the polymer allows the chain to assume a random conformation. In other words, there is no pattern to the angles that each link makes with its neighbor other than a statistical one. The fact that the molecules are in Brownian motion also leads to the concept of kinetic freedom, which is a way of saying that the chains are free to writhe in any direction. Brownian motion is temperature dependent—as the temperature increases, the movement of the molecules and their subunits becomes more and more frenetic. Conversely, as the temperature decreases, the activity of the molecules slows until, finally, at a temperature dependent on the particular rubber in question, it ceases altogether and any force that is exerted on the rubber meets the resistance of the covalent bonds linking the atoms, probably bending rather than stretching them. A rubber at the temperature of liquid nitrogen is Hookean and is said to be glassy. The temperature at which this phenomenon occurs is called the *glass transition temperature*.

At normal temperatures the rubber chains are writhing in Brownian motion. It is this writhing that produces the tension. Imagine that you hold one of these writhing molecules by the ends and try to pull it straight. You are trying, by doing work on the molecule, to decrease its entropy. If the temperature increases and the molecule writhes more violently, it opposes your efforts with greater force. As we shall see, short stretches of molecules of biological elastomers demonstrate this behavior. The rest of the molecules support and isolate these small sections. Thus biological elastomers are only partly rubbery (implying that lengths of their molecules are capable of random movement; the remainder are organized and stiff). Biological elastomers (resilin, elastin, abductin, gluten, and doubtless others) have about the same stiffness

(1 MPa) as rubber made from the latex of *Hevea brasiliensis*, but their ultimate strain is only about a fifth that of the cured (cross-linked) latex (depending on the degree of cross-linking). Biological elastomers are more complex still, since they are associated with water, which itself seems to contribute to the elastic mechanism.

1.3.1 VISCOELASTICITY: STRESS, STRAIN, AND TIME

Many biological materials contain crystalline components. A few contain rubbers that are sufficiently well cross-linked to be analyzed in terms of rubber elasticity. But by far the greatest number, if not all, biological materials are viscoelastic to a greater or lesser extent. They have a viscous component. Thus although the mechanical properties of crystalline materials and “ideal” rubbers, at constant temperature, can be described in terms of stress and strain, the mathematical description of viscoelastic materials involves the introduction of a new variable—time.

Viscoelasticity and related phenomena are of great importance in the study of biological materials. Just as strain can be measured in more than one way, so the related rate of strain (i.e., the amount of strain per unit time) can be measured in a number of different ways (Ward 2004). Cauchy strain rate is given by $dl/L_0 dt$; Hencky strain rate by $dl/l dt$. In each expression, dl is the infinitesimally small extension achieved during the short time dt , L_0 is the length at zero time, and l is the length just before the present extension.

Viscosity, η , is defined as the ratio of shearing stress to velocity gradient (Newton’s law). Its equivalence to the shear modulus can be seen in Eq. 1.9; its definition is

$$\eta = \frac{F/A}{dv/dy}, \quad [\text{Eq. 1.9}]$$

which can be compared with the expression for the shear modulus, G :

$$G = \frac{\tau}{y} = \frac{F/A}{dx/dy}. \quad [\text{Eq. 1.5a}]$$

“Newtonian” viscosity is independent of strain or shear rate. This means that if the force applied to a Newtonian fluid is doubled, the shear rate will also be doubled. Non-Newtonian fluids are those that respond with a more or less than doubled shear rate, depending on whether they show shear thinning or shear thickening. Most biological materials show shear thinning, so that doubling the force will more than double the shear rate, thus making deformation of the material relatively easier at higher shear rates. The units of viscosity are $\text{kg m}^{-1} \text{s}^{-1}$ or Pa s .

At this point it is necessary to point out that viscoelasticity is not plasticity, with which it is often confused. A viscoelastic material will return to its original shape after any deforming force has been removed (i.e., it will show an elastic response) even though it will take time to do so (i.e., it will have a viscous component to the response). A plastic material will not return to its original shape after the load is removed. In metals, plasticity is called *ductility*. It is, if you like, the converse of elasticity in that the energy of deformation is not stored but is entirely dissipated. A material can show a combination of elasticity and plasticity, in which case although

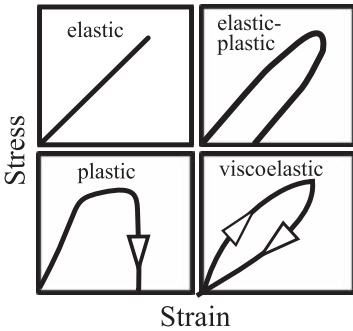


Figure 1.6. Stress–strain curves illustrating different types of behavior.

it partly returns to its original shape on removal of the load, some permanent deformation or “set” remains owing to plastic deformation or molecular “slippage” of an irreversible nature (figure 1.6).

Two major types of experiment are performed on viscoelastic materials: transient and dynamic. Transient experiments involve deforming the material (by simple elongation or in shear) and following the response of the material with time. There are two transient experiments. In one the material is loaded and the change of deformation with time is noted. This is the creep experiment. Under load, segments of the molecules of the material rotate and flow relative to one another at a rate controlled by the viscosity of the material, the stress, the temperature, and the time for which the material has been stressed. Figure 1.7 shows how the strain varies with constant (engineering) stress over a wide range of times after loading. The parameter J , obtained by dividing the strain by the stress, is the *compliance* (roughly the inverse, or opposite, of stiffness) and is here further defined as the creep compliance, $J(t)$. A compliant (or pliant) material is a nonstiff or soggy material. As a practical example, a retro-vinyl buff will choose a pickup cartridge with a “high-compliance” stylus mounting; the stylus presents minimum resistance to being moved by the irregularities that constitute the signal on the groove of the record. The molecular origin of the various regions of the compliance curve is discussed in section 1.5.

The other transient experiment is the stress-relaxation experiment, in which the material is deformed, and the force required to maintain the deformation at a constant value is measured as, with time, the molecules of the material move relative to one

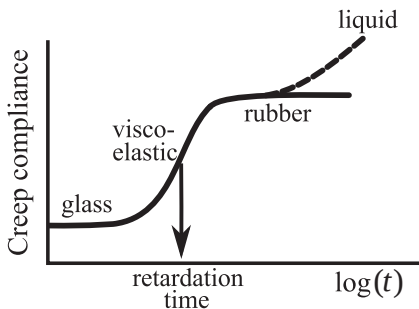


Figure 1.7. Creep compliance, $J(t)$, as a function of time, t . The characteristic or retardation time is ρ .

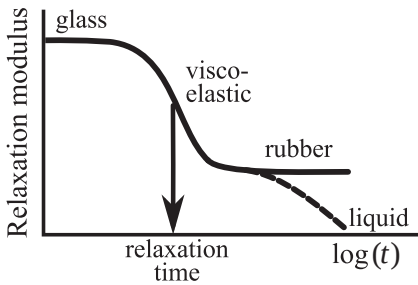


Figure 1.8. Relaxation modulus, $G(t)$, as a function of time, t . The characteristic or relaxation time is ρ .

another. Thus the stress required to hold the material at constant deformation dies away with time and is said to relax. Figure 1.8 shows how the stress varies with constant (engineering) strain (the relaxation modulus, $E(t)$ for simple extension, $G(t)$ for shear) in a manner analogous to that for creep compliance. Note that the two transient experiments are possible because there are three variables—stress, strain, and time. It is therefore possible to plot a three-dimensional surface showing how these variables are interrelated.

The other major type of experiment is the dynamic one, in which either stress or strain (usually strain) is varied cyclically (usually sinusoidally for mathematical convenience) with time, and the response is measured at various different frequencies of deformation.

Transient experiments are usually easier to understand and will be described first. The assumptions made about the mechanical response of the material are similar for both transient and dynamic experiments.

There are three major ways of describing viscoelastic behavior, all interrelated. The first starts with the Boltzmann superposition principle and is sometimes called the *integral* representation of linear viscoelasticity because it defines an integral equation. The second way, which leads to a linear differential equation and is therefore called the *differential* representation, uses assemblages of (Hookean) springs and (Newtonian) viscous elements (dashpots) as models. The third method is based on assumptions about the molecules themselves. At this point you may find it easier to read the section on the behavior of the molecules of viscoelastic materials and then come to the phenomenological approach that follows. Either way you will need to read the following sections several times to see how all the different measurements and ideas fit together.

I cannot emphasize too much that both the integral and differential models are only models and are not explanations. A number of papers on biological materials interpret the behavior of the material solely and finally in terms of springs and dashpots, as if that were an answer. The models are like the hangman's noose—they serve to concentrate the mind but not much more. With the use of mathematical expressions derived from consideration of the models it is possible to derive constants that can be used as a basis for comparison or prediction; but it is highly unlikely that a biological material can be described in terms of a single spring-and-dashpot unit. The other major caveat in the theory of viscoelasticity that follows is that both the models and their mathematical representations rely on linearity of response of both elastic

and viscous components. This is normally considered to be attainable only at strains of less (usually much less) than 0.01, but nearly all biological materials (and most artificial polymers) are not only nonlinear in response but normally function at high and extremely high (0.5+) strains. The models for viscoelasticity are not valid under these conditions. This is a severe limitation and one that is not commonly recognized. Thus much work on artificial and natural polymers is of dubious value, because it applies linear, small-strain models to nonlinear, large-strain materials. That such data may well often be internally consistent is no argument for the acceptance of the linear interpretation; it may merely be coincidence. The mathematics of viscoelasticity at large strains remains to be worked out.

1.3.2 LINEAR VISCOELASTICITY

1.3.2.1 THE INTEGRAL MODEL

The Boltzmann superposition theory may be stated as follows:

1. The creep in a specimen is a function of the entire loading history.
2. Each increment of load makes an independent and additive contribution to the total deformation.

(For creep, substitute stress-relaxation to cover all circumstances.) The first condition could be called the memory function: the response of the material is influenced by what has happened to it so far, so that it is “remembering” deformations long past and allowing them to influence its present behavior. The second condition states that if a specimen is loaded and is creeping under load, then the addition of an extra load will produce exactly the same additional creep as if that total load had been applied to the unloaded specimen and the specimen allowed to creep for the same amount of time. This is said to be a linear (i.e., directly additive) response. The second condition also implies that when the load is removed, the recovery in length of the specimen will follow the same time course as, and be identical with, the initial creep response. The importance of Boltzmann’s principle to the study of viscoelasticity is not so much that it provides any explanations as that it provides a starting point for mathematical models that can be tested against reality and refined to give a better fit. For instance, many papers have been written in which the effects of different sequences of stressing or straining have been calculated according to the Boltzmann principle and the results tested by a variety of experiments on real materials. The mathematical formulation of viscoelastic behavior derived from the Boltzmann principle is illustrated by figure 1.9.

The total strain at time t is given by

$$\varepsilon(t) = \Delta\sigma_1 J(t - \tau_1) + \Delta\sigma_2 J(t - \tau_2) + \Delta\sigma_3 J(t - \tau_3), \quad [\text{Eq. 1.10}]$$

where J is the compliance of the material, and $J(t - \tau_n)$ is the creep compliance function and is the first explicit introduction of time into these equations as an extra variable. This equation can be generalized to give

$$\varepsilon(t) = \int_{-\infty}^t J(t - \tau_n) d\sigma(\tau_n), \quad [\text{Eq. 1.11}]$$

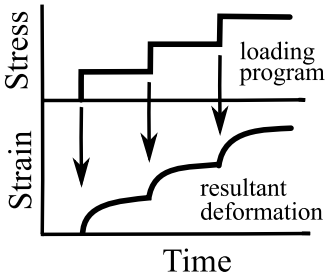


Figure 1.9. Creep behavior of an ideal linear viscoelastic solid.

which is usually rewritten—the immediate elastic response, ε , is removed—allowing mathematicians to rewrite the integral in what they find a more acceptable form:

$$\varepsilon(t) = \left[\frac{\sigma}{G_u} \right] + \int_{-\infty}^t J(t - \tau_n) \frac{d\sigma(\tau_n)}{d\tau_n} d\tau_n, \quad [\text{Eq. 1.12}]$$

where G is the immediate or unrelaxed stiffness.

This operation divides the equation into a time-independent and a time-dependent (the integral) function. The stress-relaxation modulus can be calculated in the same manner to give

$$\sigma(t) = [G_r \varepsilon] + \int_{-\infty}^t (t - \tau_r) \frac{d\varepsilon(\tau_r)}{d\tau_r} d\tau_r. \quad [\text{Eq. 1.13}]$$

Notice in particular the pattern and symmetry of Eqs. 1.16 and 1.17. This implies that there is probably a formal relationship between the two expressions, but not only is this relationship rather too simple and generalized to be of much use when dealing with biological materials, it is more easily approached from a different starting point!

1.3.2.2 THE DIFFERENTIAL MODEL

Probably the best starting point, and certainly the one most easily appreciated by most biologists, is that of mechanical models using springs (elastic elements) and dashpots (viscous elements)—the differential approach. The springs are Hookean and the dashpots Newtonian. The Maxwell model (figure 1.10) has two elements:

$$\sigma_1 = E_m \varepsilon_1 \text{ for the spring, and} \quad [\text{Eq. 1.14a}]$$

$$\sigma_2 = \eta_m d\varepsilon_2/dt \text{ for the dashpot.} \quad [\text{Eq. 1.14b}]$$

Equation 1.14a can be divided on both sides by dt and rewritten as

$$\frac{d\sigma_1}{dt} \cdot \frac{1}{E_m} = \frac{d\varepsilon_1}{dt},$$

and Eq. 1.14b can be rewritten to give

$$\frac{\sigma_2}{\eta_m} = \frac{d\varepsilon_2}{dt},$$

The two elements are in series, so that $\sigma_1 = \sigma_2 = \sigma$. Also, the total strain on the model, ε , is the sum of ε_1 and ε_2 . Thus Eqs. 1.14a and 1.14b can be added to give

$$\frac{d\sigma}{dt} \cdot \frac{1}{E_m} + \frac{\sigma}{\eta_m} = \frac{d\varepsilon_1}{dt} + \frac{d\varepsilon_2}{dt} = \frac{d\varepsilon}{dt}. \quad [\text{Eq. 1.15}]$$

In a stress-relaxation experiment the length is held constant, so

$$\frac{d\varepsilon}{dt} = 0 \quad \text{and} \quad \frac{d\sigma}{dt} \cdot \frac{1}{E_m} + \frac{\sigma}{\eta_m} = 0. \quad [\text{Eq. 1.16}]$$

Then, simple rearrangement gives

$$\frac{d\sigma}{\sigma} = -(E_m/\eta_m) dt. \quad [\text{Eq. 1.17}]$$

At the start of the stress-relaxation experiment, $t = 0$, and $\sigma = \sigma_0$ is the initial stress. Integrating the last equation, we obtain

$$\sigma = \sigma_0 \exp(-E_m/\eta_m t). \quad [\text{Eq. 1.18}]$$

In other words, the stress decays exponentially (i.e., logarithmically) with a characteristic time constant $\tau = \eta_m/E_m$, so that

$$\sigma = \sigma_0 \exp(-t/\tau). \quad [\text{Eq. 1.19}]$$

The Kelvin or Voigt model (Figure 1.10) models the creep test and, using arguments similar to those with the Maxwell model, gives rise to the expression

$$\varepsilon = \varepsilon_0 \exp(-t/\tau). \quad [\text{Eq. 1.20}]$$

Note again the extreme symmetry between the two expressions for stress-relaxation and creep.

But why use two models? The Maxwell model is no use for modeling creep, since under constant load the dashpot will allow viscous flow, and the spring will be in constant tension. All that will then be observed is the Newtonian nature of the fluid in the dashpot. This does not accord with observation of real creep experiments, so the Maxwell model is inappropriate for their description. An even more serious objection arises against the use of the Voigt model for stress-relaxation experiments, since under such conditions the model behaves as an elastic solid. We can overcome these objections by combining the two models into a standard linear solid model (figure 1.10). However, one can go on for ever with more and more complex combinations

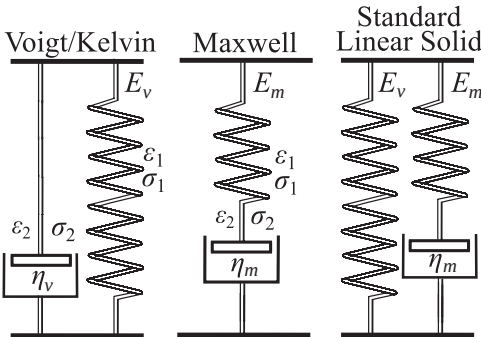


Figure 1.10. Simple spring and dashpot models.

of units that do not produce any more unifying concepts. Further developments along these lines are ignored here.

The most profitable approach with spring-and-dashpot models, at least in the modeling of artificial polymers, has been found to be that of combining numbers of Maxwell or Voigt elements (not mixing them) to obtain a spectrum of time characteristics. If the course of relaxation of a single Maxwell element is plotted, it is found to have the general shape shown in figure 1.11. If the slope of this curve is plotted against $\log$ (or $\ln$) time, a curve of shape similar to a skew log-normal distribution is obtained (figure 1.12). The vertical axis of figure 1.12 is labeled $-H(\tau)$, which is known as the *relaxation spectrum function*. The relaxation spectrum is the skew normal curve, and the relaxation time, τ , of the Maxwell element that generated it is given by the mode of this curve. If more Maxwell elements, each with a different time constant, τ , are arranged in parallel, it is not difficult to see that the decay of stress will be spread over a longer period as a result of a broader spread of relaxation times (figures 1.11 and 1.12). The peculiar usefulness of this relaxation spectrum is that it can be derived from different types of experiments and so is a convenient transform for general comparisons between materials and tests. The other usefulness of the relaxation spectrum (and, it should be added, the related retardation spectrum calculated in a similar manner from creep data) is that it gives some idea of the number and nature of the relaxation processes going on while the stresses are relaxing. This is because each process has its own characteristic relaxation time (section 1.5). In general, biological materials have a very broad relaxation/retardation spectrum, but the mesoglea of two sea anemones, *Calliactis parasitica* and *Metridium senile*, has a retardation spectrum dominated by a process having a retardation time of $10^{3.4}$ s (figure 4.16). The model used was a single Voigt element working at strains much greater than 0.01 (3, in fact), so at least in terms of strain, the model was probably inappropriate (Alexander 1962). Bill Biggs (unpublished) reworked Alexander's data and found that they were better fitted by a five-element model with retardation times in the range

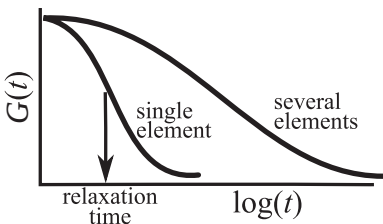


Figure 1.11. The time course of relaxation of a single Maxwell element and of several elements with a range of relaxation times.

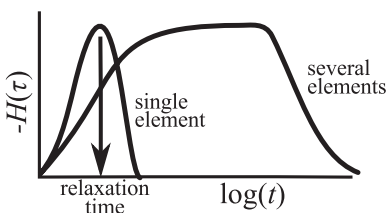


Figure 1.12. Relaxation spectrum function, $[-H(\rho)]$ derived from the curves in figure 1.11 using the Alfrey approximation (Eq. 1.26).

of 1 to 10^4 s. But on plotting $-H(\tau)$ against $\ln t$, Biggs found that the compliance is dominated by processes that have a retardation time of $10^{3.5}$ s. Although even here, where definitely more than one process is involved, a single process is dominating the response. This is very unusual with biological materials; therefore, anemone mesoglea could be a good model medium for investigating the mechanisms controlling the retardation spectra of biological materials.

The relaxation spectrum is clearly an important measure of viscoelastic behavior. Its mathematical derivation is as follows: for stress-relaxation at constant strain a single Maxwell element gives $\sigma(t) = E_n \varepsilon \exp(-t/\tau_n)$. For a number of such elements joined in parallel, all at strain ε , the stress is

$$\sigma(t) = \varepsilon \sum^n E_n \exp(-t/\tau_n), \quad [\text{Eq. 1.21}]$$

where E_n and τ_n are the stiffness and relaxation time, respectively, of the n th element. Equation 1.21 can be rewritten as

$$\sigma(t) = [G_r \varepsilon] + \varepsilon \int_0^\infty f(\tau) \exp(-t/\tau_n) d\tau. \quad [\text{Eq. 1.22}]$$

The term $G_r \varepsilon$ is the instantaneous stress. The integral represents the way in which the stress dies away with time, to give $a(t)$. The function $f(\tau) d\tau$ replaces E_n and defines the concentration of Maxwell elements with relaxation times between τ and $(\tau + d\tau)$. The relaxation modulus is then given by

$$G(t) = G_r + \varepsilon \int_0^\infty f(\tau) \exp(-t/\tau) d\tau. \quad [\text{Eq. 1.23}]$$

The “relaxation time spectrum” $f(\tau)$ is replaced by $H(\tau)$ on a logarithmic time scale (simply because a log time scale is more convenient to handle). Then

$$G(t) = G_r + \int_0^\infty H(\tau) \exp(-t/\tau) d(\ln \tau). \quad [\text{Eq. 1.24}]$$

In other words, the modulus at time t after the imposition of the strain is the sum of the initial modulus (initial stress divided by the [constant] strain) and of a function that describes how τ varies with time after the start of the experiment. Because τ is the ratio of the (Newtonian) viscosity to the stiffness of the individual elements, the integral can be considered as a function of modulus with time and describes the way in which the modulus changes (diminishes) with time. To calculate $-H(\tau)$ simply, the Alfrey approximation is used. This assumes that $\exp(-t/\tau) = 0$ up to time $t = \tau$, and $\exp(-t/\tau) = 1$ when τ is greater than t , and thus replaces a set of exponentials with a set of step functions. Equation 1.20 can then be rewritten as

$$G(t) = [G_r] + \int_{\ln \tau}^\infty H(\tau) d(\ln \tau), \quad [\text{Eq. 1.25}]$$

so that

$$H(\tau) = - \left[\frac{dG(t)}{d \ln t} \right]_{t=\tau}, \quad [\text{Eq. 1.26}]$$

which is the negative slope of a plot of relaxation modulus against $\ln$ (or $\log$) t .

1.3.2.3 THE MOLECULAR MODEL

The third major approach to understanding viscoelasticity is the molecular one. It is probably more convenient in this approach not to use stress-relaxation experiments or creep experiments but, rather, to use dynamic tests . This is not to say that transient experiments are of limited use. Far from it. Their versatility can be increased through variations in the temperature, and this variation will be referred to again once it has been dealt with in conjunction with dynamic tests. The strengths of stress-relaxation and creep tests are their ease of execution—measurement and experimental apparatus are very easily managed—and their immediate applicability to the life of the animal or plant. But rather more can be accomplished with dynamic testing, since it is more versatile and covers a wider range of conditions. The theory is also applicable to a wide range of test rig geometries.

Once again the argument is for linear viscoelastic solids. (The usual subterfuge if you have a nonlinear solid [as are most biological materials] is to say that if you deform the material by a sufficiently small amount, then the material will give a linear response.) Dynamic testing is particularly suitable for tests under such limitations. The sample is subjected to strain varying sinusoidally with time at a frequency ω . If the material being tested is Hookean, then the stress will be proportional to the strain—figure 1.13 shows stress and strain plotted against time; figure 1.14 (left) shows stress plotted against strain, which is a straight line. But if the material is viscous and has no elastic component, the stress in the material will be highest at the highest strain rate. Because the strain is varying sinusoidally about zero, the highest strain rate will be at zero strain. Stress in the material will be lowest at the lowest strain rate, which will be the point at which the strain is highest. The resulting stress–strain Lissajous figure will be a circle. Looked at another way, the stress in a viscous material induced by sinusoidal strain is proportional to the changes of accelerations

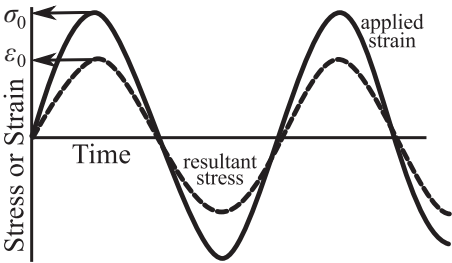


Figure 1.13. Sinusoidal strain and resulting stress induced in an elastic material.

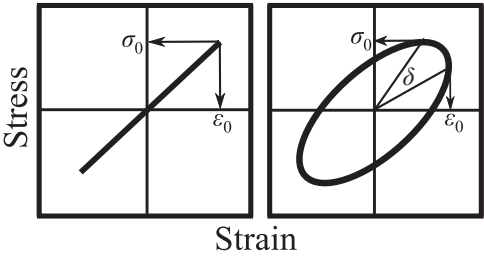


Figure 1.14. Simple Lissajous diagrams showing (left) an elastic material (as figure 1.13) and (right) a viscoelastic material (as figure 1.15).

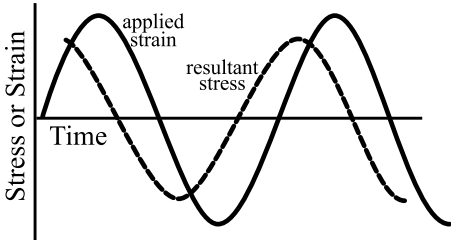


Figure 1.15. Sinusoidal strain and resulting stress induced in a viscoelastic material.

in strain and is therefore the first differential. Thus if $y = \sin x$, then $dy/dx = \cos x$. But $\cos x$ lags $\sin x$ by 90° , so the Lissajous figure is a circle.

A viscoelastic material has a response that is partly viscous and partly elastic, so its response to a sinusoidally varying strain (figure 1.15) will be a combination of the preceding two extremes (figure 1.14, right). The problem is how to extract the information from the Lissajous figures. Essentially it is possible to measure a “modulus” at the highest strain and the highest strain rate—these moduli are the elastic (or real, or storage) modulus and the viscous (or imaginary, or loss) modulus. Because the modulus, G^* , is the ratio of maximum stress to maximum strain, then

$$\sigma_0 = \epsilon_0 G^* \sin(\omega t + \delta). \quad [\text{Eq. 1.27}]$$

This expression can be expanded to give

$$\sigma_0 = \epsilon_0 (G^* \cos \delta) \sin \omega t + \epsilon_0 (G^* \sin \delta) \cos \omega t = \epsilon_0 G' \sin \omega t + \epsilon_0 G'' \cos \omega t, \quad [\text{Eq. 1.28}]$$

where G' is the elastic modulus, and G'' is the viscous modulus. There is thus a simple relationship between G^* , G' , G'' , and (δ) , which is summarized as a vector diagram (figure 1.16). The same argument can be used to extract a complex compliance, J^* , and to resolve it into its components.

The viscosity, η , can, as would be expected, be extracted from the preceding analysis very simply:

$$\eta' = G''/\omega; \quad [\text{Eq. 1.29a}]$$

$$\eta'' = G'/\omega. \quad [\text{Eq. 1.29b}]$$

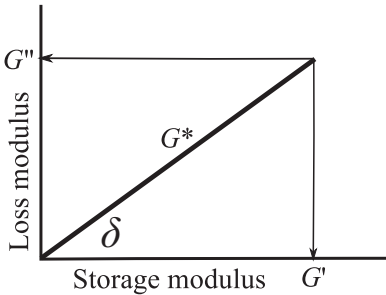


Figure 1.16. Geometric resolution of the complex modulus, G^* , into its component real (G') and imaginary (G'') moduli, and δ .

The in-phase or real component of the viscosity, η' , is often called the dynamic viscosity.

Another form of dynamic experiment that has been very useful in the investigation of biological materials will be mentioned in passing. Using either a torsion pendulum or a beam of material mounted at one end only (Ward 2004) it is possible to subject the material to oscillations that decay freely with time. In such free decay the amplitude of the oscillations decreases exponentially with time. Thus if θ_n is the amplitude of the n th oscillation,

$$\frac{\theta_n}{\theta_n + 1} = \exp \Delta, \quad [\text{Eq. 1.30}]$$

where

$$\Delta = \pi G''/G' = \pi \tan \delta \quad [\text{Eq. 1.31}]$$

Δ is also known as the logarithmic decrement and is an extremely useful experimental handle for the simple determination of G' and G'' , especially at low frequencies up to about 10 Hz in a number of different test geometries.

1.3.3 SPECTRUM OF VISCOELASTIC BEHAVIOR

It is now necessary to draw together the mathematical descriptions of viscoelasticity to show how they are interrelated, how they can complement one another in the investigation of biomaterials, and how their results relate to the structure of the biomaterials at the molecular level. The response of a polymer to dynamic oscillations is probably the easiest to understand from the molecular point of view. The most basic variable with dynamic experiments is time. In this case it's the frequency of oscillation, which is inversely proportional to time after loading in a transient experiment. The polymer molecules are in Brownian motion, just as described for rubber. The backbones are constantly changing their shape, rapidly at short range but with the entire length of the molecular writhing more slowly representing a long-range average of the short-range motions. Any side groups are wagging and twisting. To a first approximation the proportion of molecular displacements that are in phase with the externally applied oscillations represents energy storage; the proportion that are out of phase represents energy dissipated as heat. The material can exhibit the mechanical properties of a glass either if it is cooled (the amount of cooling required depends on the material and is typical of it) or if oscillations are applied at such a high frequency that essentially no backbone motions occur during the period of oscillation. The effect of either of these treatments is to restrict the amount by which the molecule can respond to the external forces by changing its shape, and so the forces are concentrated onto the backbone of the molecule. Under these conditions the molecule behaves much as a Hookean solid.

Rubbery behavior is typical of the plateau zone (figures 1.8 and 1.9). When the polymer molecules are excited at these intermediate frequencies, they become entangled very easily, much as a ball of wool carelessly handled, and the entanglements act as labile cross-links, effectively transmitting the forces.

Between these two zones—the glassy and rubbery plateaus—exists a viscoelastic transition zone. As the frequency of imposed oscillation increases from the rubbery state, the configurational changes in the network strands fail progressively to adjust themselves in the time allowed by the frequency of the oscillations. The long-range motions, being of lower frequency, are the first to run short of time in which to adjust, leaving increasingly shorter range motions to respond as the frequency rises. The strain in response to the applied stress gradually diminishes, and G' increases from the rubbery modulus of 1 MPa to the glassy modulus that is nearly 10 GPa.

What is happening to the loss modulus during these changes? G'' is a measure of the energy lost through “viscous processes.” Relatively little energy is lost while the period of oscillation is not similar to the characteristic times that describe the rates of molecular processes involved in mechanical deformation. In the rubbery and glassy zones the oscillation period is different from these molecular resonances, so losses, and G'' , are relatively small. But in the transition zone the period of oscillation is similar to that of one or other of the molecular movements; the molecular movements lag the imposed oscillation, dissipating large amounts of energy and giving a high loss modulus, thus contributing a greater viscous component. Obviously, if there are several distinct molecular movements, then there will be distinct discontinuities or secondary transitions, and the curves of G' and G'' will be rather more sinuous.

At the other end of the frequency range—the terminal zone of the modulus curve—entanglement slippage can occur within the period of oscillation, and the molecules can assume any and all possible shapes. There is thus little restraint on the material, and if it is not cross-linked, it will behave as a liquid of high viscosity. The terminal or flow zone will not appear if the material is cross-linked, and the modulus recorded will be the equilibrium modulus of a stress-relaxation experiment: the relaxation modulus, $G(t)$, is approximately a mirror image of G' reflected in the vertical axis. The appearance of the zones is also affected by the molecular weight: if the molecular weight is low (below 10 kDa), the plateau zone is absent, and the transition and terminal zones blend directly. Highly crystalline or glassy polymers will have a relatively high modulus over the entire frequency range, although there are still changes in the modulus that can give much information.

You will remember from the models of transient experiments that the relaxation and retardation times fall in the zone between the rubbery and the glassy states (figures 1.11 and 1.12; Eqs. 1.24–1.29). The characteristic relaxation processes are the same as those occurring in the transition zone of dynamic experiments. In other words, the relaxation times can be associated with the various modes of motion of the molecules. It is this basic association of molecular and mechanical properties that makes t such an important and general constant and that makes the relaxation spectrum, $H(\tau)$, such a useful form of comparison between tests, whether they be transient or dynamic. Using the Alfrey approximation to derive $H(\tau)$ from $G(\tau)$, we can derive $H(\tau)$ from G' or G'' by the following relationship:

$$H(\tau) = \left(\frac{dG'}{d \ln \omega} \right)_{1/\omega = \tau} = \frac{2}{\pi} G''_{1/\omega = \tau}. \quad [\text{Eq. 1.32}]$$

These transitions can be detected by another parameter, $\tan \delta$. Because G'' increases relative to G' in the transition regions, $\tan \delta$ will also increase in these regions. However, since G' and G'' both increase with frequency, it is not very easy to compare them visually. The ratio between the two gives a much more sensitive comparison, amplifying the differences and making them very obvious. $\tan \delta$ is therefore a much-used indicator of the presence, position, and relative magnitude of transitions. As would be expected, τ and $\tan \delta$ are closely related. For the Maxwell model, $\tan \delta = 1/\omega\tau'$; for the Voigt model, $\tan \delta = \omega\tau$. These relationships are, however, too simplified and formal to be of practical use in most instances.

So far, temperature has not been mentioned except in reference to the glassy state. A polymer tends to the glassy state either as the temperature is reduced or as the experimental time gets shorter. Thus the high-frequency parts of the dynamic experiments and the first parts of transient experiments (the first fraction of a second—assuming that the loading is instantaneous) produce results equivalent to lowering the temperature in experiments with a longer time constant. Conversely, higher temperature is equivalent to longer times in transient and dynamic experiments, bringing the polymer into a region of lower modulus. For this reason, applying heat to a glassy polymer (e.g., Perspex) softens or melts it, and the plastics-molding industry is made possible. This equivalence of time and temperature has been enshrined in the WLF (Williams, Landel, Ferry, the authors of the paper in which it was derived) equation of time–temperature equivalence or superposition. Its mathematics is beyond the present scope. This relationship allows experiments performed at different temperatures with different time constants to be related to a continuous spectrum of response, which has several implications. The first is that although the different types of transient and dynamic tests are limited in the time ranges over which they are most effective, these ranges can be extended by judiciously varying the temperature. Although the practical range of temperature for biological materials is little more than from 0°C to 40°C, even this amount can extend the time range by four to five orders of magnitude. Thus, although the experimentally convenient time scale for a transient test is about 10^0 to 10^3 s, use of the time–temperature interchangeability allows the range to be extended, from 10^{-2} to 10^5 or so, allowing the analysis of a system with values of t between 10^0 and 10^3 s. Thus the power of the transient experiment in practice becomes greater, which is a Good Thing, because transient tests on the whole require less capital outlay in equipment. However, a note of caution should be sounded. Time–temperature superposition theory has proved its usefulness with artificial polymers but has not yet been adequately tested with the much more complex biological materials. In addition, time–temperature superposition is valid only when no new relaxation processes are made possible by the change in temperature. It is possible, for instance, that a particular relaxation process could occur only above a particular temperature as a result of chemical change due to temperature.

In addition, most biological materials are hydrated. The interactions of these materials with water will change with temperature, so the WLF equation cannot be used directly. The time–temperature relationship highlights another problem that has scarcely been touched on. If a change in temperature, such as might be expected in nature, can change the reaction of a material to mechanical stimuli to the extent

suggested previously, then one might expect mechanical tissues to be adapted to the temperature at which they function. This might be true for collagen, and it is certainly true for elastin.

Water has other effects. It is a plasticizer that swells and softens biological materials. This softening can be attributed to the increase of free space within the material, which allows the polymer molecules greater kinetic freedom. Another way of saying that a material has been softened is to say that G' and G'' have been shifted to higher frequencies or lower temperatures and that the brittle dry materials that become soft and pliable on wetting are brought out of the glassy phase as the glass transition temperature is lowered by the addition of water, which acts as a diluent. The water in proteins has a much greater effect than merely altering the glass transition temperature: being a polar substance, water also greatly influences the conformation of proteins. Hydrophobic amino acids will tend to clump into zones that exclude the water, making the protein globular. Such hydrophobic interactions are largely beyond the scope of this book but are very important in controlling conformation and molecular mobility. In general, the role of water in the mechanics of biological materials has not received very much attention and could do with much more investigation.

1.4 Yield and Fracture

One of the principal characteristics of biological materials and structures is their toughness and resistance to rupture. Skin and wood are as tough as the best man-made materials, although only for wood do we have any idea why this should be so. Toughness is an important requirement for most biological materials—if bladders went pop with the regularity that boilers do, we should all be in deep trouble. There are two main ways in which a material can react when it is extended beyond its safe elastic limit. It can break immediately, or it can undergo plastic deformation, which is known as *plasticity* (in metals, *ductility*). Just as the mechanical properties of a viscoelastic material vary with temperature and strain rate, so do yield and fracture (figure 1.17). Brittle failure is characterized by low strain and rupture that occurs at the highest stress reached. Common brittle materials are biscuit, dessert jello, high-carbon steel, and the membrane around a hen's egg just beneath the shell. Brittle materials are not common in nature. Also shown in figure 1.17 is ductile failure

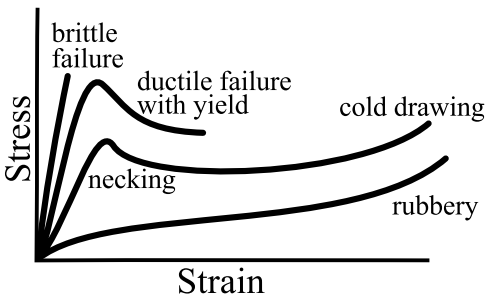


Figure 1.17. Range of yield and fracture behavior of a polymer at different temperatures. As the temperature increases, the initial modulus drops and the material stretches farther.

with yield just before failure. The yield involves plastic deformation. In the curve illustrating necking and cold drawing, the material has yielded, after which the cross section is reduced quite abruptly. The material continues to extend, with the polymer molecules reorientating themselves in the necked region at more or less constant force (although the stress is really increasing, because the section area of the sample is decreasing). With a polymer, this process of “cold drawing” produces a material, with its molecules now in a preferred orientation, that is much stiffer than the amorphous material from which it was derived and which is therefore called *strain hardened*. The strain hardening contributes to the stress–strain curve at a point later on in the curve where all the material has been cold drawn, causing a final upturn before brittle failure. A curve of this shape is also produced by hair, and the explanation is somewhat similar. Rubbery behavior is illustrated for comparison. Yield and plastic deformation are thus associated with molecular transitions; indeed, necking is itself a transition.

Most biological materials have resistance to necking and yield built into their mechanics. This property can be demonstrated as follows: the true stress (section 1.2) in a strained specimen is higher than the engineering stress based on the cross-sectional area of the unstrained specimen. The true stress–strain curve can give the ultimate tensile stress using the Considère construction (figure 1.18). The tangent to the stress–strain curve drawn from -1 on the strain axis gives the maximum stiffness of the material. Beyond the point at which the tangent touches the curve, the true stress is dropping and the material is failing. Figure 1.18 shows this process for a material with a convex stress–strain curve. Nearly all biological materials have a concave stress–strain curve to which such a tangent cannot be drawn. Thus there is no possibility of yield within the working strains of the material, which would not be so were the stress–strain curve more like that of rubber (figure 1.19). This means that even if the materials are working near or in their transition zone the energy fed into the material as it is extended will be spread evenly throughout, and there will be little possibility of local increases in stress that, as shall be explained, can lead to the failure of the material at low overall loads.

The fracture behavior of both polymers and the more complex biomaterials is relatively unknown and unexplored, especially since it usually occurs at high strains, which are mathematically difficult to characterize. The fracture of brittle materials, the foundations of which were laid by the theory of Griffith (1921), is much better

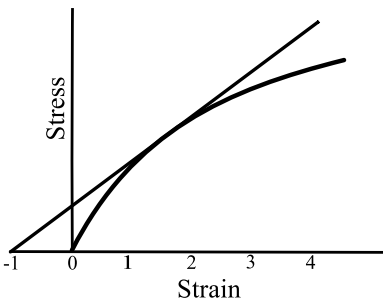


Figure 1.18. The Considère construction as a criterion of liability to yield.

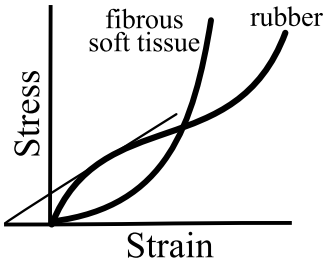


Figure 1.19. Comparison of the stress–strain curves of rubber and a typical fibrous soft biological material.

understood. Although the theory is not difficult, it is minimally applicable to polymers in the viscoelastic and rubbery regions of their behavior and is of limited use with complex composites such as bone or wood. Essentially, Griffith said that a fracture results in the formation of two new surfaces on each side of the crack and that the formation of these surfaces requires energy. This energy is stored as changes in bond length throughout the rest of the material as it is stretched. The process of fracture then involves the transmission of this energy to the fracture surfaces, at the same time relaxing the strain in the area from which energy has been released. If the crack is considered to be linear, traveling at right angles to the direction of the applied stress (figure 1.20), then it is reasonable to suppose that the energy that the crack is absorbing comes from an area defined by the crack as the diameter of a circle. It is fairly obvious that as the crack (length L) extends (by an amount Δl), the amount of energy available for the propagation of the crack will increase at a greater rate, since it is proportional to the square of the crack length. Up to a certain crack length the energy released from this area is not enough to propagate the crack, but after this point (the *critical* or *Griffith* length) more energy is released than is required, so the crack is propagated (figure 1.21). This mechanism, plus the capacity to transmit the stresses to the crack tip where fracture is occurring, accounts for the fracture of brittle (non-ductile) materials. But ductility or plasticity (i.e., irrecoverable deformation) can use

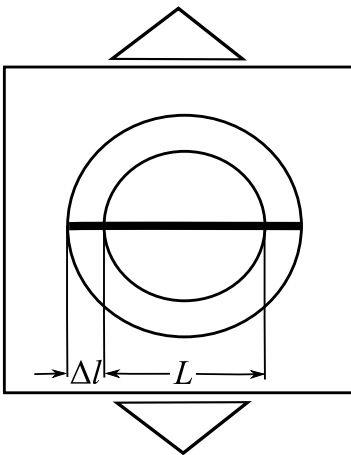


Figure 1.20. Model for deriving the general conditions for the propagation of a crack.

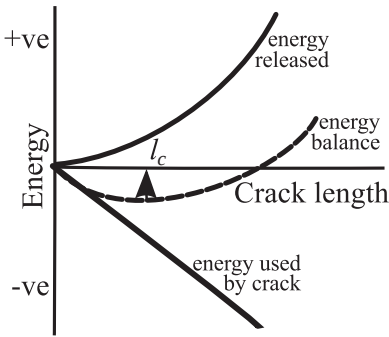


Figure 1.21. The energy conditions associated with the propagation of cracks showing the derivation of the critical crack length, l_c . The term “+ve” indicates strain energy released by the crack; “-ve” indicates energy used to make new surfaces as the crack progresses.

up energy before it can reach the crack tip to contribute to the new fracture surface. In metals, ductility can account for far more energy than is needed for propagation of the crack, and this has also been shown to be true for brittle fracture in polymers. In more compliant polymers and complex materials such as skin, this behavior is expressed in motions such as the alignment of the polymer molecules ahead of the crack tip.

The realization that two criteria have to be fulfilled for a piece of material to break arose largely from Griffith's work. The strength of the material (the critical stress intensity, K_C) must be reached, and there must be sufficient elastic strain energy (work of fracture) available at the tip of the crack to propagate it. In engineering, the critical stress intensity is known as the *toughness*: engineers are, on the whole, more interested in how to resist the initiation of a crack, whereas biological materials sometimes seem to encourage the formation of a crack and then control its propagation through work-of-fracture mechanisms.

In most of the attempts to produce a theory of fracture, one of the aims has been to produce a material parameter that does not depend on the shape of the specimen or the orientation of the crack. This is a reasonable aim that has substantially been achieved for linear materials, in which all the strain energy is involved in fracture and in which fracture occurs at relatively small strains. But one of the properties of polymers at large strains that has already been mentioned is the tendency for the molecules to become oriented in response to the deforming forces. This orientation can, in some materials, lead to strain crystallization somewhat akin to the strain hardening of a cold-drawn polymer. Such crystallization can be reversible and so be a function of strain. Thus for such materials the problem arises that its morphology changes with strain. And just as the properties of the strain-hardened polymer are different from those of the random polymer, so, too, the properties of any polymer may be considered to change with extension. In fact, a cross-linked polymer in a state of large static strain, at equilibrium, may be considered as a new anisotropic material whose linear viscoelastic properties can be studied. It should therefore be no surprise that there is (probably) no unique work of fracture for high-strain polymers.

The other complication is that energy can be dissipated at sites remote from the fracture surface. (Oliver Wendell Holmes's “The Deacon's Masterpiece; or, ‘The Wonderful One-Hoss Shay,’ a Logical Story” (Holmes 1907) demonstrates this property

to perfection. All the knocks of everyday life were accumulated within the perfectly balanced and adjusted structure and kept away from any fracture surface until, exactly a hundred years after it was made, the shay collapsed into a heap of dust because it could absorb no more strain energy. A few early fiberglass car bodies were rather like this.)

Some indication of this dissipation can be obtained from hysteresis tests, where the hysteresis results from energy lost within the bulk of the material. Should one take account of this loss when calculating the fracture constants? For filled rubbers, in which the amount of filler (carbon black) is varied, it is found that not only is this loss directly correlated with the degree of hysteresis but that higher hysteretic losses are associated with greater toughness. And in the compressive failure of wood across the grain, the size and distribution of large vessels control the distribution of weakness and orchestrate the uniform failure of the material (Hepworth et al. 2002); a similar mechanism seems to be invoked in bone by microcracks (Reilly and Currey 2000). Work with self-healing materials (Trask, Williams, and Bond 2007) shows that a limited amount of damage actually makes material tougher. Additionally, it may well be that mechanisms for energy dissipation also vary with strain and strain rate. A further factor is the transmission of strain energy to the crack tip, where it is needed to supply the energy for crack propagation. The effectiveness of the transmission of stresses to the developing crack tip is a function of shear modulus, which itself changes with shear rate. Thus the speed at which the crack is fed with energy will depend on the rate of change of shape in the material around the crack.

Toughness can be increased by a number of mechanisms, all of which increase the amount of energy required for fracture and all of which can be present in a tough material. The following are some of them:

1. The strain energy is unable to reach the crack tip. For instance, it can be dissipated by plastic yield and failure of the material remote from the crack. It is quite possible that viscous effects within the material will slow down the rate of delivery of energy to the crack tip, so that the crack can be propagated only slowly and with difficulty. Transfer of fluid from one site to another within the material falls into this category and seems to be a mechanism for toughening teeth (Fox 1980) and, very probably, other biomaterials. The strain energy may not be transmitted at all if the shear stiffness of the matrix material is too low (evidenced by a J-shaped stress-strain curve, common in soft tissues) (Mai and Atkins 1989).
2. The total energy required for cracking is raised. For example, the fracture surface is very convoluted and therefore of large area, or the material at the crack tip deforms plastically.
3. The stress at the crack tip is defocused by, for example, increasing its radius of curvature or by the Cook-Gordon effect (see figure 5.29). The sharpness of the crack tip governs the stress intensity. It focuses the strain energy onto the next susceptible bond. At high strains in unidirectional extension, the crack tip rounds off into a semi-circle. In rubbers (and probably in other high-strain materials) there is evidence of strain crystallization at the crack tip (now a semicircle) that further strengthens this most vulnerable area.

4. As the crack opens, fibers or filaments extend across it, dissipating energy by their own deformation (Fantner et al. 2005) or by friction as they pull out from the bulk of the material (Pisanova et al. 2001).
5. The material is prestressed in the sense opposite that in which it is most likely to be loaded (e.g., in compression if the most likely loads will be tensile), so that a crack cannot start until this prestress is paid off.
6. The entire structure is so small that the strain energy necessary for fracture cannot be stored.

In practical terms, fracture toughness can be measured and calculated in a large number of different ways. For each test geometry there is a specific mathematical solution that makes a number of assumptions about the material and the test and allows calculation of toughness from a number of more or less simple measurements. General information is available from the work of Atkins (Atkins and Mai 1985). However, biological materials frequently transgress these assumptions, being anisotropic or very stretchy or inhomogeneous or oddly shaped. There is a pragmatic way of coping with these problems: use a test in which the crack grows in a stable fashion such that the test piece can be unloaded (i.e., returned to its original length or shape, or until the recording device shows that no load is still being applied) before it breaks into two pieces. An example is the double cantilever beam (figure 1.22). If a sample of this shape is displaced as indicated, it will, when it reaches breaking load, start to fracture (figure 1.23). If the fracture is allowed to propagate with increasing displacement, and the test piece is unloaded before the specimen breaks in two, the force–extension curve will follow the path *MNO*. Notice that this is not a stress–strain curve: the lines

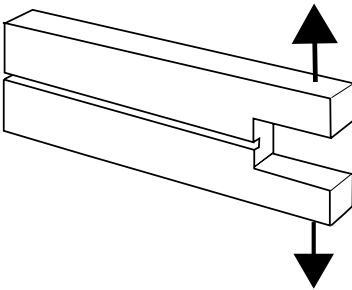


Figure 1.22. Notched double cantilever beam used for the generation of the data in figure 1.23.

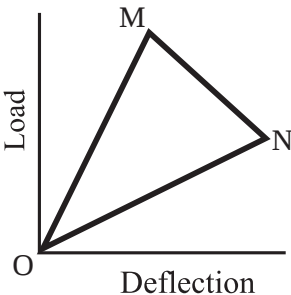


Figure 1.23. Load deflection curve for the estimation of fracture toughness using the Gurney work-area approach.

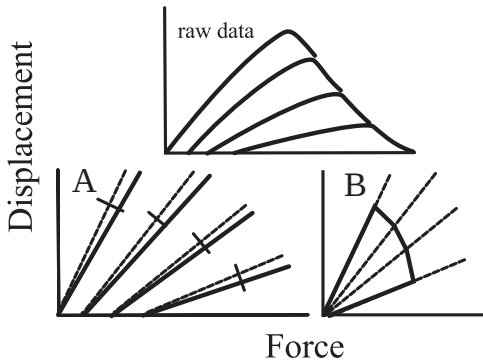


Figure 1.24. Process for deriving work-area curves from experimental data. The slopes and point at which fracture starts (the force drops) are extracted and plotted (A), and a machine stiffness correction is applied (dotted lines). The plastic component of fracture is taken out by moving all the lines to the origin of the graph (B), and the elastic fracture energy is given by the new enclosed areas (Jackson, Vincent, and Turner 1988).

MO and *NO* differ in slope because the cross section of the test piece has been reduced by the cracking. Notice also that because the test piece has been unloaded and returned to zero load, the area within the triangle *OMN* represents the work done, or energy absorbed, in fracturing the specimen. In a viscoelastic or elasto-plastic material there are additional losses due to hysteresis or permanent deformation on loading and unloading (figure 1.24). Corrections for extrinsic factors such as the stiffness of the test machine can be made graphically (Jackson, Vincent, and Turner 1988).

This type of test can be performed with morphologies other than the double cantilever beam: so long as the crack propagates in a stable fashion and can be arrested at the will of the experimenter, it will give results that can be analyzed in this fashion. Atkins and Mai (1985) give the criteria for controlled cracking in a number of morphologies. Alternatively, if the test is performed at a sufficiently slow rate of extension such that the force falls to zero just as the crack finishes traveling across the specimen and it breaks completely, then there can be no strain energy left in the material, and the area under the curve represents only the energy used for fracture. This is a rather risky trick and implies that you can talk to your sample in its own language! Either way, any elastic strain energy is discounted from the final reckoning, and the energy that the force–deformation curve encloses is that required to propagate the crack. The great advantage of this graphical technique is that it is entirely independent of any mathematical model and the assumptions involved in generating such a model. As such, it is particularly useful for biological materials, which are so complex that there frequently isn't a respectable mathematical theory to describe or analyze their fracture processes. This general approach is known eponymously as the *Gurney work-area method* (Gurney and Ngan 1971). (I was once giving a lecture on the fracture mechanics of food, a class of material that comes in so many difficult shapes and sizes [mostly small] that this method is one of the few that works, since it has no prerequisites. At the end of the lecture an elderly gentleman who had been sitting in the front row of the lecture theater came up to me and said, "I'm so pleased you like my ideas"!)

There is another problem. Any piece of tissue will have a number of imperfections (scratches, nicks, notches, and cuts) whose size, nature, and distribution are difficult to control or predict. Depending on the nature of the material, these imperfections

can affect, or even direct, the mechanisms of failure; for instance, they can initiate a crack. This is because any imperfection has the effect of concentrating stress around it, more especially at sharp corners (Gordon 1976). Smooth corners and edges are important in controlling fracture. One strategy is to confine the deformation to a very small area, effectively limiting failure to a small zone, as is achieved in the “trouser-tear” test and by techniques involving cutting or wedging. Another strategy is to introduce an imperfection larger than any of those already in the test piece. This is commonly done by notching or cutting the specimen. This option is not available with brittle materials, in which the starter crack need be only a few micrometers long and thus more or less uncontrollable. In that case a statistical approach is necessary, such as that of Weibull (1951). This approach assumes that the strength of the material is distributed more or less normally about a mean value and provides the mean and the deviation (the Weibull modulus). This has proved useful in the analysis of the fracture properties of potato crisps/chips (Rojo and Vincent 2008, 2009).

The problems involved in fracture of biological materials when they are stretched in two directions at right angles to each other (biaxial straining—the preceding discussion has been concerned with uniaxial straining only) have not been investigated much, even though they are of greater relevance to organisms. It seems likely that fracture of a biaxially strained specimen will be more “brittle” than fracture of a uniaxially strained specimen. Compare the way in which a balloon pops when pricked with a pin with the way a piece of rubber from the same balloon reacts to the same stimulus when stretched by the same amount but uniaxially. The “toughness” of rubber is dependent on the way in which that toughness is measured. Thus it is quite possible that the toughness of skin will vary with its position on the body in accordance with the direction and magnitude of likely strains.

1.5 Adhesion

Adhesion will be mentioned only briefly in that the proper tools for its measurement have been discussed in general terms already. There are a number of different mechanisms of adhesion (Kinlock 1987), which can be summarized as mechanical interlocking (micrometer-sized roughness); diffusion of one component into the other (which has been observed when two artificial rubbers are pressed together); electron transfer (an arcane and mostly insignificant theory and effect); and adsorption (by van der Waals forces and hydrogen or chemical bonds). In general, the strength of an adhesive bond is probably best thought of as a problem in fracture, so it can be measured with the caveats of fracture mechanics in mind. As an example, the adhesion of the gecko’s foot relies on its being peeled off the substrate rather like a piece of sticky tape pulled up from one end. Gordon (1976) gives a good discussion of adhesive joints—both their formation and strength or toughness—in his treatment of how wooden aircraft were built and maintained.

Proteins

Proteins are polymers of amino acids; polysaccharides are polymers of sugars. Between them, these two groups of substances make up nearly all the skeletal tissues, pliant and stiff, of animals and plants. The precise complement of amino acids or sugars and the order in which they are arranged along the polymer chain ultimately control the mechanical properties of the material that they form. These chemicals and the materials they form are the subject of this and the next three chapters. Proteins and polysaccharides of mechanical significance can be divided into two main groups—fibrous and space-filling. In general, proteins are more important as fibers, represented mainly by collagen. Polysaccharides are just as capable of forming fibrous materials, but their major function seems to be stabilization of water in many biomaterials. The reasons for this dichotomy of function are not clear, and there are probably many. One reason may have to do with the ease with which hydrophobic interactions—important in stabilizing interactions in an aqueous environment—can be formed by the two classes of polymers, and with the amount of water that can be stabilized. The main difference is probably the diversity and number of bonds that hold the monomer units together: polysaccharides can be linked in many ways and can form branching polymers, whereas amino acids are stuck—literally—with the peptide bond, so proteins are linear molecules.

Notwithstanding these differences, an important general aspect of both groups of polymers is the way the linked monomer units (sometimes called *residues*) interact with one another, both as neighbors within or alongside a polymer chain and cooperatively as interacting lengths of polymer. Ultimately, it is these interactions that give the polymer its properties and dictate the reaction of the polymer to mechanical challenges in a given environment. Many mechanical tests designed to probe the nature of such materials do so by varying the conditions of temperature, humidity, solvation, or chemistry around the bonds and observing how the response to mechanical challenge varies.

2.1 Amino Acids and Their Polymerization—Primary Structure

One way of approaching the mechanical properties of proteins is to consider the bond-forming and space-filling properties of the amino acids and then to examine what structures they take part in and why (Dickerson and Geis 1969; Rayment 2001). The basic structure of an amino acid is shown in figure 2.1. The central carbon atom is called the α -carbon; R may be one of many possibilities (the most common are

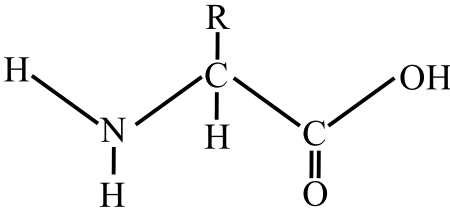


Figure 2.1. The basic structure of an amino acid. Commonly found side (prosthetic) groups (R) are shown in figure 2.2.

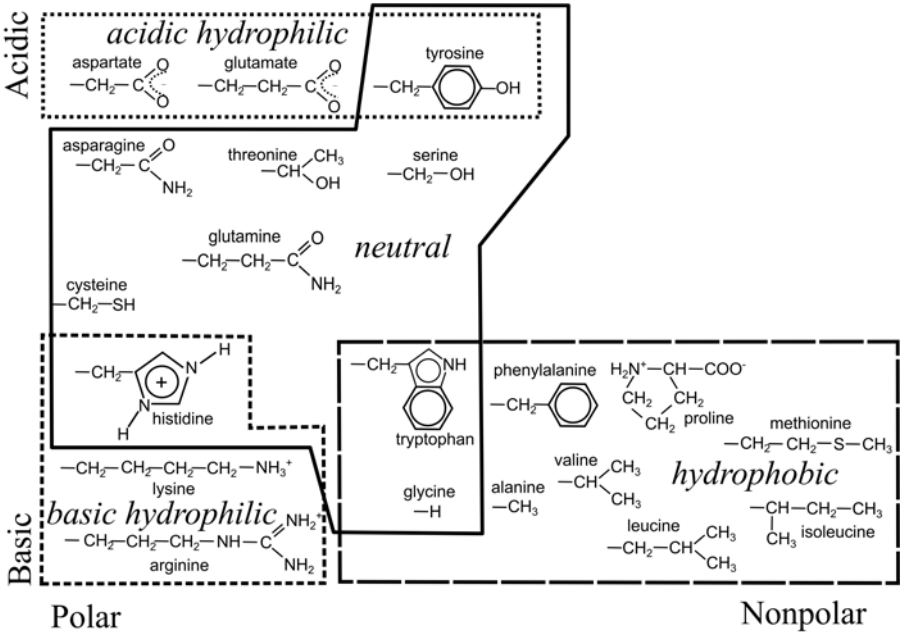


Figure 2.2. The range of prosthetic groups found most commonly on biogenic amino acids, grouped according to broad chemical properties.

shown in figure 2.2). Amino acids are joined together or polymerized through the peptide bond or amide link that is formed in a condensation reaction (figure 2.3, which leads to the bonding shown in figure 2.4) during protein synthesis in the ribosome. Although figure 2.4 shows a double bond between the carbon and the oxygen and a single bond between the carbon and the nitrogen (the amide bond), in reality the two bonds average out to give a low-energy double bond like a cloud arcing from the oxygen to the carbon to the nitrogen. The effect of this bonding is to hold the amide group in a single plane, a virtually constant form for the peptide bond. This means that almost no rotation is possible about the peptide bond, but there is freedom of rotation about the single bonds between the amide groups and the single α -carbon atoms, which allows the polypeptide chain to assume a large number of configurations. The actual number of configurations possible can be shown to be limited by the following basic considerations:

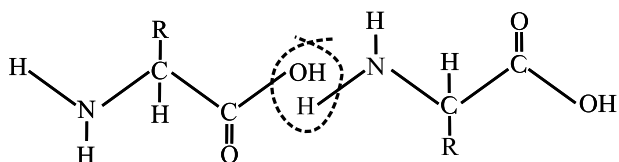


Figure 2.3. Polymerization (condensation) of two amino acids.

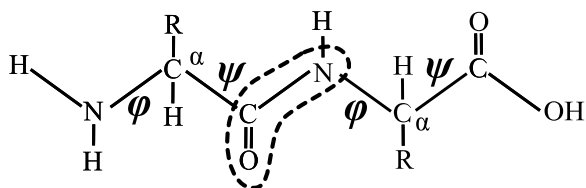


Figure 2.4. The linkage that holds the amide group in a single plane, and thus restricts rotation about the main chain.

1. The shapes of the backbone allowed by the limited rotations about the bonds to the α -carbons.
2. The limitations on (1) set by the size of the side group, R
3. The differing types of stabilizing secondary bonds (notably hydrogen bonds) formed between various oxygens and hydrogens (notably those associated with the peptide bond).
4. The chemical nature of the side chains and the interactions of these groups with one another and with the immediate chemical environment.

The polypeptide chain has two degrees of rotational freedom per residue: the twist about the α -C—N bond axis (ϕ) and the twist about the α -C—C axis (ψ). A list of all the (ϕ, ψ) values for all the residues will completely define the shape of the chain. The permissible values for ϕ and ψ can be calculated to lie within certain bounds defined by the minimum distance to which the unbonded atoms of the peptide bond can approach. Some values of ϕ and ψ are not permitted because the unbonded atoms of neighboring groups would approach too closely. If the values of ϕ and ψ at every α -carbon are the same, the chain naturally falls into a helical shape. The number of amino acids per turn and the distance between the turns (the pitch of the helix) are both determined once ϕ and ψ are specified.

Obviously, parameters of such general importance as ϕ and ψ , whose values can be derived from purely theoretical considerations, can be used to show which regular shapes or conformations of the peptide chain are possible. Ramachandran and his colleagues did this at Madras (Ramachandran et al. 1963). A conformation map or “Ramachandran plot” for the polypeptide chain is shown in figure 2.5. The region from $\phi = 20^\circ$ to $\psi = 140^\circ$ is particularly favorable and corresponds to a rotation of the N—H bond toward the side chain and the removal of the CO group of the same peptide as far away as possible from the side chain. Within this region

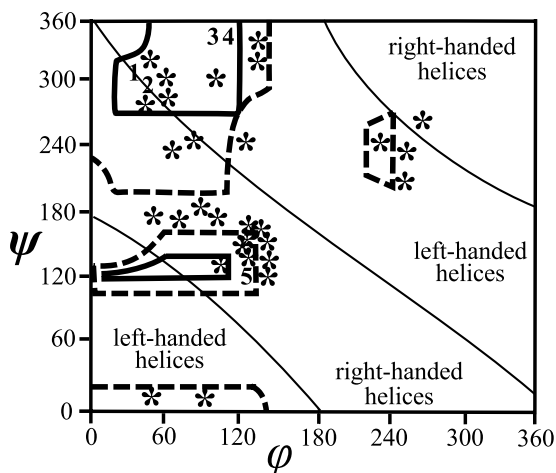


Figure 2.5. Ramachandran plot for a polypeptide chain (see text).

(marked) are the antiparallel (1) and parallel (2) β -pleated sheets, the polyproline helix (3), the collagen coiled coil (4), and the α -helix (5) (numbers refer to figure 2.5). Slight variations on these angles, which depend on the context of various parts of the protein chains, can be plotted on this diagram (asterisks). These various conformations are secondary structures; the primary structure is the sequence of amino acids.

Three major points must be made about these regular structures. They are stabilized by interactions between the hydrogens of the NH groups and the oxygen of the C=O group. These hydrogen bonds are of great importance in the selection of the most stable conformation. Several helices are possible when hydrogen bonding occurs between the C=O of one peptide link and the NH of the second, third, or fourth successive peptide link. In the most favored helix—the α -helix—the hydrogen bonds form between one peptide link and the third following link, making the bonds particularly unstrained and stable. This helix is the most stable and most commonly found. The stability of such helices (and other regular structures) is a function of their size—that is, the number of residues contributing to the structure. In the α -helix, for example, the protein chain, originally kinetically free, is arranged to form one turn of the helix, allowing the formation of one H-bond. But with two turns of the helix, four H-bonds are formed; with three turns, eight H-bonds are formed; with n turns, the number of H-bonds formed is $[3.6n - 2.6]$, or the integer just below this number. Thus the longer the helix, the more stable it becomes, owing to the cooperative interaction of many local forces, and thus achieves long-range order. This is obviously only a first approximation, as will be apparent when other interactions involved in the formation and stabilization of secondary structure are considered. Finally, the side group can affect both the rotational freedom of the bonds in the peptide link and the closeness of packing of the amino acids.

2.2 Conformation—Secondary Structure

Computer analysis of the areas of proteins and amino acids making up the different conformations shows that close correlations can be made between conformation and the occurrence and position of specific amino acids. These correlations have been studied in model systems of polypeptides made with a single type of amino acid. The conformational correlations obtained from the study of native proteins, which are far more complex, seem mainly to be confirmed by these studies. The implications are then threefold: First, the control of protein conformation must largely be a localized affair. If there were a significant contribution from more remote amino acids, as might occur if two parts of the protein were folded close to each other, then such a good correlation would not be possible. Second, the protein must assume its final configuration irrespective of the conformations that it adopts beforehand (e.g., during synthesis or some renaturation process). But if each individual residue of an unfolded chain could exist in only two states (a gross underestimate), then for a chain of 150 residues there would be 10^{45} possible conformations. If each conformation could be assumed with the frequency of a molecular rotation (10^{12} s^{-1}), then it would take about 10^6 years for all possible conformations to be assumed. It takes about two minutes for a protein such as lysozyme to be synthesized and to assume its native conformation, so there must be a limited number of conformational pathways for the protein to take toward the assumption of its native conformation, which will be a conformation with relatively low free energy. The determinism of biology, which underpins the logic of the subject, thus states that natural selection (with thermodynamics dictating the folding) yields an amino acid sequence that forms a biologically useful molecule, presumably with a limited number of pathways from the unfolded form to a unique native structure of low free energy. All these assumptions must be taken to be true for a single type of environment of the protein (e.g., aqueous with a particular pH and salt concentration) so that a protein can be secreted, by a cell, in one conformation, acted on by a different environment outside the cell, and assume another conformation. This mechanism is the basis of so-called self-assembly systems of the extracellular matrix. Cellular products can be designed to have specific and variable reactions with other extracellular components and thus build up tendon, bone, cuticle, and the like. The third implication of these correlations is that it is possible to predict something of the conformation of a protein simply from its amino acid sequence.

It has thus been found possible to assign amino acids to various categories according to their helix-forming tendencies (table 2.1). There are several factors that determine the conformational preference of a given amino acid residue. For instance, the most likely state for a protein chain in the absence of any stabilizing factors is irregular. This is the conformation of highest entropy. Therefore, an amino acid that does not have any factors that are favorable to the formation of α -helices must necessarily be a helix breaker. This argument seems to hold for glycyl residues. They have no side chain and so have no energetic factors favorable to the formation of helices; thus the entropy of irregular zones makes glycyl residues helix-breaking. When a $\beta\text{-CH}_2$ group is added (giving alanine, abbreviated A—see table 2.1), the resulting

TABLE 2.1
Biological amino acids

<i>Amino acid</i>	<i>Abbreviations</i>		<i>Helix former</i>	<i>Helix neutral</i>	<i>Helix breaker</i>
<i>Aliphatic</i>					
Glycine	Gly	G			X
Alanine	Ala	A	X		
Valine	Val	V	X		
Leucine	Leu	L	X		
Isoleucine	Ile	I	X		
Proline	Pro	P			X
<i>Aromatic</i>					
Phenylalanine	Phe	F		X	
Tyrosine	Tyr	Y		X	
Tryptophan	Trp	W	X		
<i>Polar uncharged</i>					
Serine	Ser	S			X
Threonine	Thr	T		X	
Cysteine	Cys	C		X	
Methionine	Met	M	X		
Asparagine	Asn	N			X
Glutamine	Gln	Q	X		
<i>Positively charged</i>					
Lysine	Lys	K		X	
Arginine	Arg	R		X	
Histidine	His	H	X		
<i>Negatively charged</i>					
Aspartate	Asp	D			X
Glutamate	Glu	E	X		

possible interactions favor helix formation. But if a CONH_2 group is then added, to give asparagine (N), the polar side chain interacts with the amide group of the peptide link, destabilizing the helical conformation. These electrostatic effects are smaller if the polar group is farther from the peptide link. Thus the insertion of an extra CH_2 group into the side chain to give glutamine (Q) results in a helix-forming residue. A similar argument applies for the series aspartate–glutamate. Aspartate has a charged group that can form H-bonds with neighboring backbone amide groups when in the random conformation, so it is a helix breaker. Glutamate has the same charged group spaced farther from the backbone by a CH_2 group and thus cannot take part in such interactions. Serine (S) is a helix breaker because it has a charged group close to the backbone, but proline (P) is a helix breaker because the rotational freedom of the $\alpha\text{-C}-\text{N}$ bond is severely restricted by the ring structure. Proline (and the related hydroxyproline) is extremely important as a constituent of collagen.

There are further considerations in the assumption and stabilization of particular conformations in proteins. The chemical nature (acidic, basic, polar, nonpolar, etc.) of the amino acids (figure 2.2) is important in determining medium- and long-range interactions. A medium-range interaction may be said to extend from one residue to a distance of, say, four residues on either side. The α -helix is thus stabilized by medium-range interactions in the form of hydrogen bonds. Long-range interactions involve other amino acids and the environment. In an aqueous environment polar side groups tend to interact with the water to a much greater extent than do the nonpolar groups, which generally keeps the polar groups to the outside of the protein and the nonpolar groups directed toward the center. Nonpolar interactions are very important in stabilizing interactions between neighboring proteins; in effect, they form “sticky” areas on the surface of proteins.

Interactions of side chains (figure 2.2) include salt linkages or electrostatic bonds (between ionic groups of opposite charge); hydrogen bonds (between polar groups); van der Waals and London forces (between nonpolar groups); and covalent disulfide links (between sulfur-containing side chains). The strength of these interactions is important in considerations of the mechanical properties of the proteins in which they occur, since these interactions largely give the material its integrity and resist the externally applied mechanical forces. Table 2.2 gives the relative strengths of these bonds. This trilogy of α -helix, β -sheet and tight or β -turns, and the collagen helix underlays the conformation of all proteins. Conveniently for us, the proteins important for imparting mechanical properties tend to have rather regular structures, probably because mechanical properties are dependent on materials that have to transmit force over distances that by comparison with the nanotechnology that drives life are very great. This set of requirements ensures that these structural polymers are moderately amenable to analysis, modeling, and copying in various ways.

2.3 Structural Proteins

There are several families of structural proteins; the main ones are keratins, collagens, silks, and elastins. These families are of differing complexities and are based on chemical and molecular rather than mechanical criteria. In the following discussion more significance should be given to these mechanisms than to the particular

TABLE 2.2
Typical bond energies

<i>Name</i>	<i>Activation energy (approx.) (kcal mol⁻¹)</i>
Covalent	30–100
Hydrogen	10–20
Ionic	1–10
van der Waals	0.1
London	0.05

class of material from which the examples are taken. Thus the keratins, typified as sulfur-containing proteins (section 2.3.1), can have α -helical, β -sheet, or mixed conformations. Collagens have a very uniform and definitive conformation, at least in the fibrous part of the molecule. Some silks contain collagen; mussel byssus thread contains moieties typical of collagen, silk, and elastin.

2.3.1 KERATINS

Mammalian keratins are a subset of a family of α -helical proteins known as *intermediate filaments* (Bernot and Coulombe 2004). Such filaments are found in muscle (Oshima 2007), hagfish slime (Fudge et al. 2003), the cytoskeleton (Ackbarow et al. 2009), and keratinous products of the dermis such as hair, stratum corneum (the outer layer of the skin), horn, hoof, and baleen. The basic building block is an antiparallel pair of α -helices coiled together (figure 2.6). There are two classes (from a total of five) of intermediate filaments that form keratins: type I (acidic keratins, about 40–60 kDa) and type II (neutral to basic keratins, about 50–70 kDa) (Bowden et al. 1994). One each of types I and II is needed to form a filament, and since there are at least 25 different versions of each, and since all filaments have preferred partners, that gives a minimum of 25 types of keratin filament. This is quite a selection to choose from, so we should expect some very varied properties, both mechanical and morphological. The α -helices are the smallest component of a hierarchical system. They are assembled into larger units that form ropelike structures (Hearle 2000), or protofibrils. But there are also β -keratins, typical of reptiles (and birds), which are made of a twisted form of β -sheet protein (Fraser and Parry 1996, 2008). Keratins are thus a varied group of proteins, found mostly in vertebrate animals, that contain significant amounts of disulfide or tyrosine-based cross-linking, both in the fibers and in the surrounding stabilizing matrix (Fraser and MacRae 1980; Gregg and Rogers 1984). It is this sulfur that gives the nasty smell when horn, hair, hoof, or feather is burned.

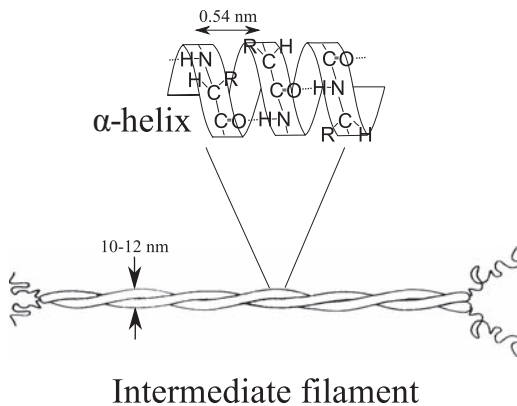


Figure 2.6. The α -helix (see figure 2.5) and the intermediate filament, one of the structures in which it is found.

α -Keratin has been most studied in hair (Rogers et al. 2006), more especially wool (figure 2.7). When wool is stretched (figure 2.8) (Hearle, Chapman, and Senior 1971), the initial Hookean part of the curve has a modulus of about 4 GPa. A higher value, approaching 10 GPa, has been measured in more or less perfect arrays of α -helices (polyglutamate) obtained by orienting them on the surface of a Langmuir-Blodgett trough (Wegner 1989). The yield point, at a strain of about 0.02, marks a change in modulus that drops by a factor of 10 or so. Such a yield or transition indicates a change in the molecular structure. The most likely explanation, and one that is supported by evidence from X-ray diffraction, mechanical tests, and molecular modeling, is that the secondary bonds that stabilize the helical structure rupture, and the helices start to unravel and form β -sheet structures (Kreplak et al. 2004). This result dates to one of the earliest experiments in this area, when Astbury, working in Leeds, United Kingdom, stretched hair and saw how the X-ray diffraction patterns changed (Astbury and Woods 1933). He called the initial structure “alpha” and the final structure “beta,” and that’s how it all started. Experiment shows that the modulus increases at a strain of about 0.3 (in the wet), at which point only about a third of the material has unraveled (Danilatos and Feughelman 1979). The mechanical behavior found in the intact hair is observed down to the lowest level of hierarchy: tensile tests of the basic keratin unit—the antiparallel pair of helices—have the same mechanical signature, demonstrating that there is no modifying structure between molecule and hair. In other words, the

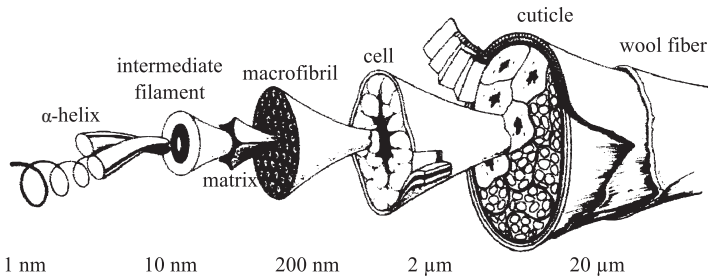


Figure 2.7. The hierarchy of hair. (Adapted from *International Journal of Biological Macromolecules* 27,. Hearle, J.W.S, A critical review of the structural mechanics of wool and hair fibres, pp. 123–138, Copyright 2000, with permission from Elsevier.)

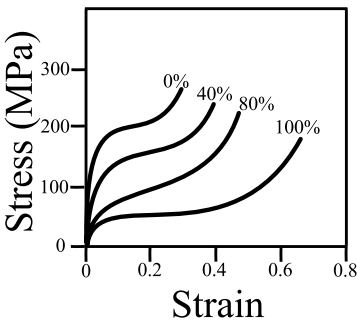


Figure 2.8. Mechanical properties of α -keratin typified by wool at different relative humidities (Hearle 1971).

microstructure of the hair has to show all the helices packed parallel to one another—which is pretty much the case (Fraser and Parry 2003).

The influence of water is also of great interest. It reduces the sustained stresses in all parts of the curve except for the initial part of high modulus, suggesting that it can't enter and solvate the α -helices, but it can help them unravel. As water enters the hair, axial swelling is small (about 1% at 100% relative humidity), and the ratio between Young's modulus and the shear modulus changes from 2.7:1 (indicative of a more or less isotropic material) to about 0.5:1 (indicating a highly anisotropic material). Indeed, application of Eq. 4.1 (section 4.3) yields the respective Poisson's ratios 0.35 (quite reasonable for a stiff material at low strains) and 0.9 (Fraser and MacRae 1980), which is much nearer that found for some soft tissues (section 4.3) consisting of uniaxially oriented fibers in a pliant phase. The inference is that water does not enter the helical portion but enters the matrix phase. The postyield phase has also been shown to involve exchange of S—S bonds within the matrix, so that whereas the fibrous phase seems to be relatively stable and crystalline, the matrix phase seems to be labile, although still organized into β structures (Fraser, Rogers, and Parry 2003). The matrix is composed of a heterogeneous assemblage of proteins (sometimes rather clumsily called keratin-associated proteins or KAPs) containing much cystine or tyrosine (Rogers et al. 2006). The pliant matrix is probably the basis of the hairstyling industry: under the influence of high temperature and humidity the H-bonds break, leaving the hair more pliable. The hair can then be held in chosen positions while the temperature and humidity return to ambient, allowing the H-bonds to re-form and hold the hair in its new, "permanently" waved shape.

There is some uncertainty about the status of water in hair, which is inevitably associated with the status of cross-linking, since the degree of swelling when the hair is wet is limited by the degree of swelling when the covalent S—S bonds were formed. This limitation is a basic result of the physics of rubber elasticity (Treloar 1975). The implication is that the S—S bonds are formed while the hair is still swollen owing to hydration within the follicle and that dehydration occurs at a later stage. By analogy with the many phenolically tanned invertebrate structural proteins (insect cuticle and mussel byssus threads are prime examples) it seems very likely that the high-tyrosine proteins are important for increasing the hydrophobicity of the matrix and driving the water out at a later stage in the development of the hair, though a search of the literature suggests that this possibility has not been investigated. Such chemistry would entail the presence of tyrosinase, which would convert the tyrosine residues to the more active quinone form and would stimulate the formation of melanin. There is another aspect of the watery origins of hair, which has to do with the self-assembly of the keratin fibrils. The molecular organization is beautifully periodic and pervasive—almost crystalline. Such organization has to be a product of the chemistry of the proteins, but not just the proteins. The proteins are in water, a medium that both gives freedom and imposes order. The freedom comes from the diluent and plasticizing effects. Dilution of the proteins allows them to move relatively freely and to adopt a number of associations, permanent or temporary. Plasticization is due to the additional effect of the occupation of the polar sites by water molecules, which masks the sites, preventing them from making contact with similar sites on

other filaments. But least understood is the response of water to the hydrophobic areas of the filaments. In the polar environment of water these areas are potential sticky patches. For the moment regard the preceding statement as a flag with the key words *self-assembly*, *hydrophobic interactions*, and *liquid crystals*. The key words are intimately (and literally) bound to one another and form a logical continuum to the entire topic of biological materials in a way that hardly exists with man-made materials. Liquid crystalline structures are explored in more detail in section 5.1.

If hair is extended and then allowed to retract, it is found to show very high hysteresis (figure 2.9) (Hearle 2000). The same experiment with horn surprisingly reveals high plasticity and very little elastic hysteresis (figure 2.10) (Trim et al. 2010). Hysteresis, though not of the magnitude seen in hair, has been found in elastomers “filled” with finely divided carbon black particles (or any other sort of small particle) and seems to be intimately associated with toughness. The argument is that energy used up in processes with high hysteretic losses is unavailable for fracture. However, beware that horn and hoof are only part of the loading chain of the vertebrate skeleton and that strength and energy absorption may not be the main functions of these materials: the prime function of both is much more likely to be the control of the propagation of fracture. Hooves stop legs from fraying at the ends; horn keratin deflects cracks that might proceed through to the bony horn core, which is what transfers the forces of fighting to the neck muscles, where they are absorbed. Under these circumstances

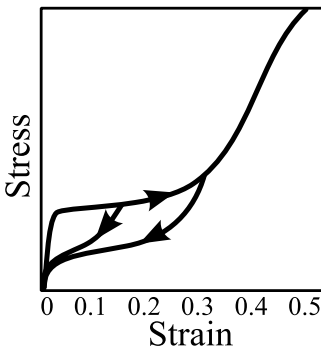


Figure 2.9. Hysteresis of α -keratin in hair (Hearle 2000).

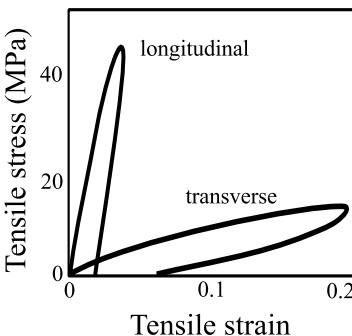


Figure 2.10. Hysteresis and plastic deformation of α -keratin in horn (Trim et al. 2010).

it may be the function of the material not to resist cracking but to deflect cracks from more vulnerable structures or materials. Application of a fiber composite model (section 5.2.4) to horn keratin gave a value of 6.1 GPa for the stiffness of the fibers, which have an effective length of about 40 nm. The stiffness of the matrix protein varied from 6.1 GPa (dry) to 0.9 GPa (wet). The morphology of horn keratin is relatively simple—it seems to be relatively easy to model and has a work of fracture of about 10 kJ m^{-2} (Kitchener 1987). This is a typical value for a high-performance composite (Harris 1980). The keratin of hoof and baleen is much more complex and hierarchical, being composed of helically wound tubes of keratin in a keratin matrix. Wet hoof keratin is relatively soft, with a Young's modulus of only about 0.5 GPa (Bertram and Gosline 1986). For a material that is galloped on, used for digging and fighting, and that cannot be instantly replaced if it breaks, toughness is obviously important.

Stratum corneum, the tough outer layer of mammalian skin, is a rather more diffuse α -keratin, with the fibers oriented more or less randomly. This orientation is reasonable if the material is to accommodate stresses from several directions. This diffuseness is reflected in the tensile test curves (Papir, Hsu, and Wildnaeur 1975) that show that although there is a yield point, it does not separate an initial region whose modulus is independent of water content from a postyield region that is dependent on water content. These findings suggest that the matrix makes an important contribution to the initial modulus as well as to the postyield modulus. The way the modulus changes with water content shows a marked change at 70% relative humidity (figure 2.11a), which could be called the onset of plasticity. This change is associated with a change in the way that water is bound into the matrix, as shown by differential scanning calorimetry (DSC). This technique measures the heat exchanges as the temperature of the sample is raised at a constant rate. If the specimen is cooled in liquid nitrogen so that all the water in it is frozen and totally glassy, then as the specimen is warmed up, any frozen water that melts will absorb a latent heat of fusion as it does so, producing a peak (endotherm) on the trace of heat absorption against time, which is the output of the DSC. If the water is closely associated with the protein (often called “bound” water), then it is less likely that it can form ice with unstrained bonds. The strained bonds in the ice that manages to form from this water will be less stable than unstrained bonds and will be destabilized at a lower temperature than those of ice formed from free water. Thus bound water tends to have a lower freezing/melting temperature than free water, and this difference can be detected by DSC. The amount of heat absorbed by the melting water gives an estimate of how much water is present in that bound fraction. With stratum corneum, DSC studies in conjunction with mechanical tests suggest that the onset of plasticity is associated with a particular fraction of the bound water—that is, water that is immobilized within the matrix in a particular way. This correlation could be due to at least two effects: the interpolation of water molecules into H-bonded linkages between the amide and carbonyl groups, which effectively breaks the H-bonds, and the provision of extra space around the side chains (indicated by the specimen's swelling), which allows more freedom of rotation about the bonds both in the main peptide chain and in the side chains. Studies with collagen and polyamides (Hiltner, Andersen, and Baer 1973) showed that water can be correlated with particular molecular relaxation processes, so these ideas seem reasonable. In each instance the

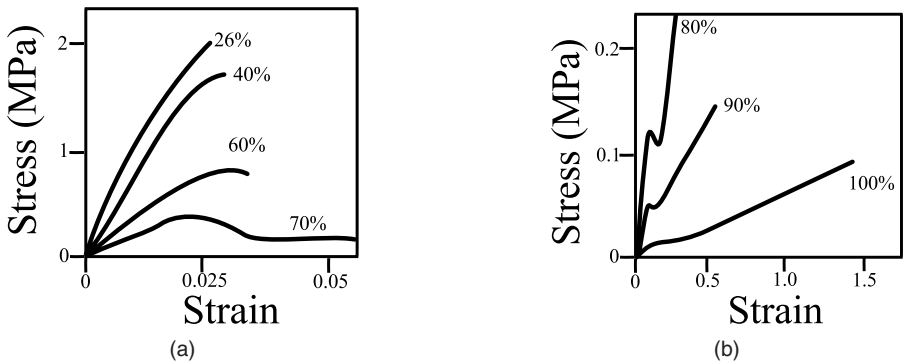


Figure 2.11 (a, b). The effect of sorbed water on the mechanical properties of stratum corneum from the skin of a newborn rat. Note the change in strain scale between figures 2.11a and 2.11b. (Papir et al. 1975).

water plasticizes the material in the same way that plasticizers soften artificial plastics such as PVC. It is interesting that somewhat similar results can be obtained with the interaction of water with nylon, an artificial polyamide (Bretz, Hertzberg, and Manson 1979). These findings once again confirm the reasonableness of the basic approach to biological materials from a physicochemical standpoint.

There have been relatively few studies on the keratins of birds and reptiles. Astbury and his coworkers (Astbury and Marwick 1932) in Leeds in the 1930s were the first to probe the molecular structure of feather keratins using X-ray diffraction. They concluded that the conformation was basically a slightly contracted form of β -sheet with an axial rise per residue of about 0.31 nm. The keratin could be strained by a maximum of about 0.06, which produced an increase in the axial rise to 0.33 nm, close to the value found in β -keratin from stretched hair.

In duck and chicken feathers all the proteins have much the same molecular weight (about 11 kDa) and amino acid composition, and when cross-reacted with antisera made to whole feather, they show only a single immunogen. Seagull and emu (O'Donnell and Inglis 1974) and chick (Arai et al. 1983) feather keratins have largely been sequenced and show remarkable similarities (Gregg and Rogers 1984). The latest model (Fraser and Parry 2008) confirms many of these results and shows a helical filament with four repeating units per turn as a common motif across several bird and reptilian species. Each repeating unit consists of a pair of twisted β -sheets (figure 2.12). Each sheet is believed to constitute a 32-residue segment of the keratin molecule, which comprises around 100 residues; the remainder make up the matrix. The surface between the adpressed β -sheets contains most of the serine and glycine residues, which pack closely and are highly hydrophobic. The outer surface of the β -sheet pair has charged and cysteine residues. Toward either end of the chain there are noncrystalline sections rich in cystine. The central crystalline portion is relatively conservative in amino acid composition and sequence, whereas the ends of the molecules are much more heterogeneous and variable. It thus seems that the fiber structure

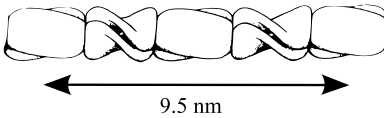


Figure 2.12. Twisted β -sheet conformation of feather keratin. (Reprinted from *International Journal of Biological Macromolecules*, 19, Fraser, R.D.B., and Parry, D.A.D, The molecular structure of reptilian keratin, pp. 207–211, Copyright 1996, with permission from Elsevier.)

is strongly conserved by evolution, whereas the matrix structure can be less specific. It therefore seems likely that each protein contributes to both the fiber (30% of its length) and the matrix (70% of its length). Feather keratin can self-assemble from extracted material in solution, forming structures indistinguishable from the natural ones (Brush 1983). The stability of their interactions is increased in an aqueous environment, which tends to cause the hydrophobic structures to aggregate. Once again, we have the makings of a system that will self-assemble in an aqueous environment with polar and nonpolar zones.

The mechanical properties of feather keratin have not been much investigated. At 5 GPa (Fraser and MacRae 1980), or perhaps even 8–10 GPa (Purslow and Vincent 1978), feather keratin is at least twice as stiff as hair, although the stiffness also depends on the fiber orientation within the feather rachis (Cameron, Wess, and Bonser 2003). This dependence is reasonable, since stresses are projected directly onto the covalent bonds of the extended β structure, whereas in hair the H-bonds that stabilize the helix absorb the initial load. These are probably fewer and certainly weaker than the covalent bonds of feather keratin, in which the H-bonds are aligned at right angles to the principal stresses and so absorb only shear loads. Feather keratin also shows much less hysteresis than hair keratin and does not exhibit any phase changes, as implied by abrupt changes in modulus. This property is certainly necessary for feather rachis, which must be able to provide a wing structure that can transfer the maximum amount of the bird's energy into displacement of air. When tested in three-point bending, feather rachis shows a Hookean response with no hysteresis, right up to the sort of deflections that occur in flight. The elasticity of feather rachis is important in flight, during which the strain energy in the rachis keeps the wing tip moving at the end of the stroke while the wing root has started on its recovery. If you have rowed in a racing shell, you know that it is easier to finish a stroke cleanly if you have a whippy blade, because you can hold the handle stationary at the end of the stroke and give yourself more time in which to start the recovery while the whip (strain energy) in the oar is still carrying the blade through the water. The action of the feather rachis at the end of the wing stroke seems to be exactly analogous (Pennycuik and Lock 1976).

2.3.2 SILKS

Feather keratin is primarily a twisted structure. Silks are also structures, but they are more planar and well extended. Other conformations occur in silks, notably some related to the α -helix and collagen proteins. The silk protein is made up primarily of

glycine, alanine, and serine with a small amount of other amino acids, mostly with bulky side chains. The amount of glycine (G) is about the same as that of alanine (A) and serine (S) combined, which suggests the possibility of a repeating structure GS or GA, with twice the amount of GA as of GS. Also note that both glycine and alanine are not very polar and that serine is only a little more polar.

The next expectation is for a repeating structure that is stabilized by hydrophobic interactions. X-ray crystallography allied with model building and the investigation of model materials [notably poly-L-(AG) and poly-L-(AGAGSG)] showed that the most probable structure is indeed the latter one. The silk of the spider *Nephila clavipes* is rather different: about 29% of the amino acid composition is alanine, and 45% is glycine. These amino acids are arranged in sequences of six or seven alanines with a (GAG) sequence at each end, and sequences in which glycines occur in pairs, as in GGYGGLGSQG. Polypeptide chains of this sequence form antiparallel β -sheets that, with a slight pleat in them, are stabilized by unstrained H-bonds (the parallel β -sheet conformation requires the H-bonds to be strained and so it is energetically less favorable and more unstable). This configuration is shown in figure 2.13. The significance of the alternating residues becomes apparent when several such chains are packed together to form a fiber. The glycine side chains ($-H$) all project to one side of the pleated sheet; the serine and alanine chains project to the other side. When the chains are packed, these groups alternate to give a very compact structure (figure 2.14) that is stabilized by van der Waals forces. The diagram in figure 2.15 summarizes the major stabilizing forces within the β -sheet areas as vectors (i.e., line length is somewhat proportional to bond strength). Thus the fiber should be stiff along the

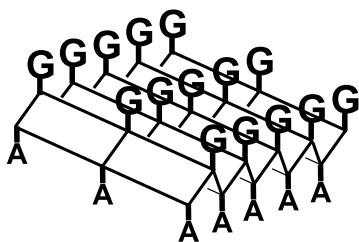


Figure 2.13. Arrangement of glycine and alanine in a silk extended β -sheet.

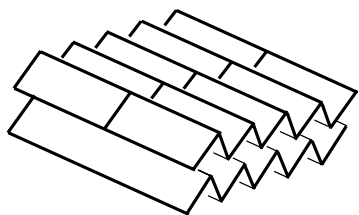


Figure 2.14. Silk extended β -sheet stacking in a self-assembling structure.

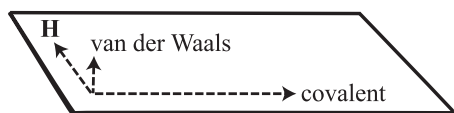


Figure 2.15. The major vectors stabilizing silk extended β -sheet structure.

length of the sheet, as indeed it is: about 10 GPa (Gosline, DeMont, and Denny 1986), which is very similar to the value for feather keratin. As in feather keratin, the H-bonds are normal to the direction in which the principal stresses act, so they stabilize the sheet. The van der Waals forces are relatively weak, so movement is possible between the sheets, resulting in a flexible fiber.

A careful look at the amino acid sequence reveals more subtlety. Crudely, the presence of repeated sequences (“motifs”) suggests a common form of polymer—a block copolymer—in which some parts of the chain are made of one component and other parts made of another. The tendency is then for the two motifs to segregate into separate zones, which can give a material with more than one phase (Ruokolainen, ten Brinke, and Ikkala 1999). This model applies to a number of biological materials, such as keratins, in which the α -helices form a crystalline phase, and the remaining parts of the same molecule, with different secondary structure, form a matrix phase. In silk the adjacent β -lengths run in opposite directions (antiparallel), with the large and the small amino acid side chains interdigitating between adjacent layers. However, the sequence (LGXQ) (where X can be S, G, or N) forms a β -turn, a well-known structure in proteins that is commonly found connecting two strands of antiparallel β -sheet. The amount of β -sheet material suggested by the amino acid sequence can be compared with estimates of crystallinity using physical techniques. X-ray diffraction indicates 9%–15% crystals of $2 \times 5 \times 6$ nm (Grubb and Jelinski 1997), and solid-state NMR shows that more than a third of the alanine is in crystalline β -sheet material, which constitutes 11% of the fiber (Simmons, Michael, and Jelinski 1996). Molecular modeling of the stiffness of the fibers suggests that the fibers should be 12% crystalline (Gosline et al., 1986), so the evidence from different sources agrees very well! The amino acids in the noncrystalline proportion are relatively bulky, tending to form disordered regions. Figure 2.16 correlates extensibility and the percentage of amino acids with bulky side groups. These disordered regions are responsible for the extensibility of silk fibers. *Antherea* silk has sufficient amorphous material to show a hairlike stress–strain curve: a yield at a strain of 0.05 followed by a low modulus region as the amorphous areas become oriented and a final high modulus region, just before break at a strain of 0.35, showing that the chains in the amorphous region have been oriented by the strain.

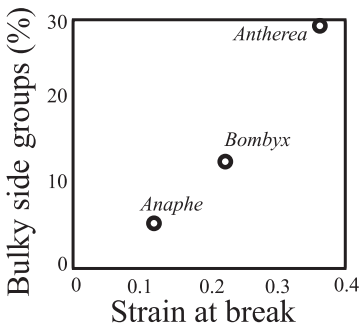


Figure 2.16. Relation between extensibility and content of bulky side groups in silkworm silk.

Dragline silk of the spider *Araneus diadematus* shortens dramatically when wetted and shows evidence of being rubbery, a phenomenon that has been ascribed to increased randomness in the amorphous areas (Gosline, DeMont, and Denny 1986). This interpretation has been challenged by Vollrath and Edmonds (1989), who argue that the effects are due simply to the increased effect of surface tension of the water. Certainly, it seems that at the small sizes involved, the forces generated at the air–water interface can be a significant component of the total. The stiffness and extensibility of the silk that a spider uses in its web are likely therefore to be controlled by three factors: the amino acid composition, which determines the amount of crystallinity possible; the distribution and amount of water in and around the fiber; and the draw ratio. The *draw ratio* is a measure of the amount by which the fiber is extended before the stabilizing secondary forces lock the polymer chains relative to one another. With silk this step presumably occurs in the presence of water, which plasticizes the material and then is expelled, leaving the silk stabilized. The average draw ratio required for silk formation in *Bombyx* is 3 as the silk passes from one end of the spinneret to the other (Iizuka 1966). A high draw ratio results in highly oriented polymer chains and a high modulus fiber; a low draw ratio gives a fiber of high amorphous content, low modulus, and high extensibility. That draw ratio is probably important has been shown with *Bombyx* silk, for which the Young’s modulus and crystallinity are inversely proportional to the diameter of the thread, and it is likely that the finer thread is produced by a higher draw ratio. The process of extruding the thread has been shown to be crucial in the production of the dragline silk by the spider *Nephila edulis*. The silk is produced through the duct of the major ampullate gland, which can be regarded as an elongated convergent die. At natural spinning speeds most of the draw-down process occurs within the lumen of this duct. Microscopy of the spinning duct suggests that this transformation involves formation of antiparallel β -sheets induced by extensional flow; this hypothesis was confirmed by experiment (D. Knight, M. Knight, and Vollrath 2000). Silk can be “spun” in vitro, where it is found not only that a minimum shear rate is required for the silk to become crystalline but that this process is helped by the presence of divalent ions such as Ca^{2+} and Mg^{2+} .

Many silks are spun underwater—spiders build webs underwater and trap air bubbles in them, and caddis larvae stick sand granules and plant matter together to make a protective (and camouflaged) tube in which to live. These silks are produced from a watery solution and spun into water—so how does the spun silk remain insoluble? There are some differences in the amino acid composition: sequences such as GX, GGX, GPGXX, and SXSXSX (where X is any amino acid) are common, and amounts of alanine [which in moth and spider silks occurs in runs of poly(A) and poly(GA)] are low. Also, unusual in moth silks, about 15% of the residues are positively charged. Part of the answer to the question of insolubility is that the serine is phosphorylated (figure 2.17) (Stewart and Wang 2010). Similar chemistry is associated with other underwater adhesives such as mussel byssus adhesive plaques and the sticky thread produced by sea cucumbers (DeMoor et al. 2003; Flammang et al. 2009; Stewart et al. 2004). The current theory is that the phosphoserine binds to Ca^{2+} ions, creating strong cross-links between the protein chains and driving out the water. This technology is known to industry: phosphate added to latex paints greatly

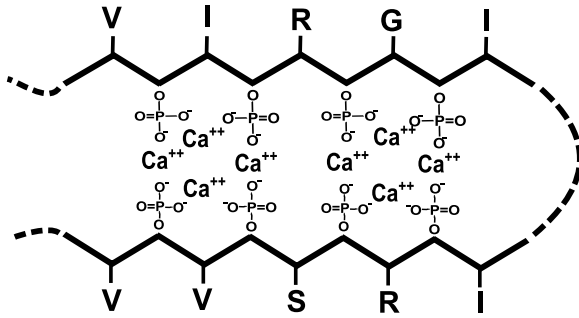


Figure 2.17. Phosphorylation of waterproof silk (Stewart and Wang 2010).

increases their wet interfacial adhesion; and if teeth are primed with polymerizable phosphates, the interfacial bond strength with dental materials is greatly increased.

2.3.3 COLLAGENS

Collagens make up a family of closely related proteins that form characteristic fibers throughout the animal kingdom (excluding Protista), and a large body of literature exists on the chemistry, structure, biosynthesis, and mechanics of collagen from various animals and in various states. Twenty-eight types of collagen and more than 30 molecular species called “collagen” have been recognized so far in vertebrates (Bächinger et al. 2010) and a much wider variety in invertebrate animals. In the most abundant collagens (types I to III) and in types V and XI, the helical region is more than 1000 amino acid residues long, forming a rodlike structure that is necessary for the formation of fibrils. These collagens are also of similar size and are collectively known as the fiber-forming collagens. Other groups are basement membrane collagens (IV); basement membrane zone collagens (XV and XVIII); type VI collagen; type VII collagen; short-chain collagens (VIII and X); FACIT (fibril-associated collagen with interrupted triple helices) (IX, XII, and XIV); FACIT-like collagens (XVI, XIX, XX, XXI, and XXII); transmembrane collagens (XIII, XVII, XXIII, and XXV); new fibrillar collagens (XXIV and XXVII); type XXVI collagen; and type XXVIII collagen. All these collagens can be associated with specific tissues and therefore functions (Burgeson and Nimni 1992). The 28 collagens are coded for by about 25 different genes, but the variety of vertebrate collagens may in fact be even greater, since the genes also can give rise to spliced variants. Unless otherwise stated, the rest of this section refers to type I collagen fibrils, which account for more than 90% of the collagen in the vertebrate body.

Collagen is the most common fibrous protein and is the basis of many glues and of the gelatin industry. Collagen occurs in most tissues and is probably the most prevalent protein in the body (table 2.3). In skin and basement membrane it occurs as a reinforcing fiber. It can also function as the winding of a pressure vessel, as in nematodes, earthworms, and sharks. In tendon and muscle collagen is concerned with transmitting tensile stresses, and it is in this form, notably as rat tail tendon,

TABLE 2.3
Distribution of collagen in a mouse

Skin	40%
Bone, cartilage	10%–20%
Tendon	25%
Blood vessels	5%–10%
Internal organs	2%–8%
Muscle	1%–2%

Note: Collagen constitutes 20% of the total protein.

that it has been most studied. The characteristic amino acid composition is almost diagnostic of collagen. In particular, hydroxyproline (proline hydroxylated after its incorporation into the peptide chain) is often used as a marker amino acid and for estimating the collagen content of tissues. The most striking feature is the large amount of glycine, proline, and hydroxyproline, which are all helix breakers, totaling half the protein. Statistically, every third residue should be glycine, making up units with proline and hydroxyproline of the form —(GXP)— and —(GX-Hyp)—, where X is any other amino acid, and Hyp is hydroxyproline. In the collagen molecule as a whole, glycine does occur at every third residue, but the rest is not quite so regular. However, the structure that the sequence forms is well within the acceptable limits on the Ramachandran plot and is a slow left-handed helix. Three of these helices coil around one another to give the collagen triple helix. Polymers of glycine, proline, or hydroxyproline also form a collagen helix, so for some reason the helix is energetically favorable for these amino acids. Having glycine at every third position is important because it enables the protein chains to come close together and form hydrogen bonds between the chains. In fact, a collagen-like molecule can be constructed from any three polypeptide chains containing glycine as every third residue. However, some “driving force” is required to push the conformation in the direction of the collagen conformation—the loss of entropy in going from an irregular conformation to a collagen triple helix would be too large in the absence of proline and hydroxyproline, which direct the conformation. There is reason to think that it is possible to have too much proline and hydroxyproline, since a collagen that was too stable might be difficult to degrade during development and the dissolution that precedes healing.

To form a fiber, the collagen molecules have to form a continuous structure with plenty of overlap that allows stresses to be passed from one molecule to the next. Just as with keratins, the basic microfibril (in this instance, tropocollagen) is assembled into increasingly larger units, producing a hierarchy of structure that can finally form such components as tendon (figure 2.18). This structure is very regular; for instance, the banding pattern seen in the electron microscope and in X-ray diffraction patterns is characteristic of collagen. This banding pattern has a periodicity of 67 nm or so (depending on the source of the collagen); the individual tropocollagen molecules are 280 nm long and pack together in the now-familiar quarter-stagger pattern. This pattern can be formed *in vitro* if the collagen is precipitated from a weakly acid

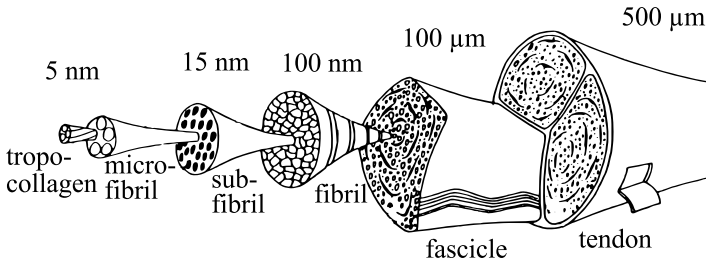


Figure 2.18. Hierarchy of collagen structure in a tendon.

solution with 1% NaCl. The pattern is therefore determined by the amino acid sequence and was confirmed by a model in which two collagen amino acid sequences were stepped past each other and the total hydrophobic and charge interactions estimated between each pair of amino acids (Hulmes et al.1973). Maxima in the strength of these interactions occur at intervals corresponding to the quarter stagger (figure 2.19). In this sort of arrangement the collagen fibril can be considered to be crystalline, since it shows a well-defined melting temperature (about 60°C) at which the fiber shrinks to about one-third its length and becomes rubbery. Thermodynamically, this is a first-order transition and is characteristic of melting in crystalline materials.

In tendon and other structures the collagen is associated with varying amounts of other proteins and acid mucopolysaccharides (see chapter 4). The matrix is involved with the transmission of shear within the tendon and hence affects properties such as toughness. However, the tensile properties of tendon can be accounted for mostly in terms of the collagen triple helix, which constitutes 70%–80% of the dry weight of the tendon. Collagen has been investigated under tension by X-ray diffraction of stretched samples (figure 2.20). The small-angle diffraction pattern, corresponding to the 67-nm banding, increases linearly with strain, accounting for 90% of the strain up to strains of about 0.15. However, the wide-angle spacing, which corresponds to the atomic spacing within the protein chains, does not increase nearly so quickly with strain, indicating that although the helix itself is being stretched, this extension can account for only about half the total strain. The conclusion from these experiments is that the tendon is (at least) a two-phase material with relatively low extensibility

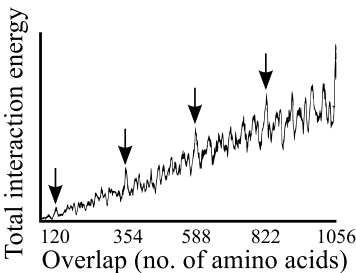


Figure 2.19. Summed interaction energy between two collagen amino acid sequences as they are stepped past each other. Arrows show local maxima that drive the quarter-stagger pattern.

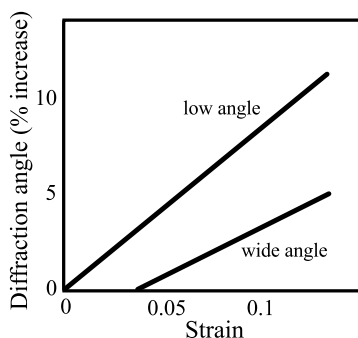


Figure 2.20. Correlation of macro- and microstrain in stretched collagen from rat tail tendon. Wide-angle data give 0.291 nm spacing; low-angle data give 67 nm spacing (Cowan et al. 1958).

in the crystalline regions and relatively high extensibility in some less organized regions. Presumably, the less organized regions are the matrix component. Removal of the polysaccharide component of the matrix makes little difference to the mechanics of the tendon; removal of the noncollagenous protein fraction reduces both the initial modulus and the ultimate strain by a factor of 10.

One further point requires consideration before we look at the mechanical properties of bulk collagen in a tendon, namely, the effect of water. Dry collagen is brittle and stiff, with a Young's modulus of about 6 GPa. This is stiffer than hair and about as stiff as feather keratin but not so stiff as silk. Addition of water softens the collagen progressively. This interaction of water has been shown to occur in a well-defined manner. The most strongly interacting water is probably incorporated as an integral part of the structure of the triple helix, probably about two water molecules for each tripeptide. When the water content exceeds two molecules per tripeptide, the molecule starts to swell laterally. At this level of hydration the water has a marked plasticizing effect and is present at about 25% (w/w). It is probably associated with the charged side chains. At greater water content, more is associated with the matrix material.

The behavior of water in association with collagen is in some ways similar to its behavior in association with nylon and stratum corneum. The stress-strain curve of materials containing collagen, such as a tendon, is very typical (figure 2.21). There is a toe region of low modulus that curves around to give a region of much higher modulus. The latter is the normally quoted modulus. The toe part is normally attributed to the unfolding of crimp structures (figure 2.18, fascicle). This interpretation has been well confirmed with both polarized light studies and mathematical analysis. The length of the toe region varies with the source of the tendon; therefore, presumably, the amount of crimping varies in different collagens. The modulus of a wet tendon in the steep part of the curve is typically 1.5 GPa (Bennett et al. 1986). This value is typical of amorphous polymers, but one would expect a higher value from a relatively crystalline material in which the tensile stress is borne directly by the main-chain covalent bonds. For example, silk can have a modulus nearer 100 GPa. One collagen that is obviously very stiff and strong is found in the wall of the nematocyst, the hydraulic capsule that powers the sting of coelenterates (sea anemones,

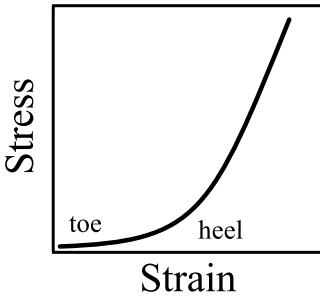


Figure 2.21. Typical stress–strain curve of a material containing randomly orientated or crimped fibers.

corals, and jellyfish). In the freshwater polyp *Hydra vulgaris*, the capsule contains a 2 M salt solution and so reaches a turgor pressure of 150 atm (15 MPa) before it shoots out the dart in the first phase of stinging. If the nematocyst is assumed to be a sphere with walls 200 nm thick, then the stress in the collagen is 190 to 375 MPa (Holstein et al. 1994). If one adds a reasonable safety factor into this design, the fracture stress will need to be about 500 MPa. If this collagen stretches over the straight part of the “normal” collagen stress–strain curve, as seems reasonable for a fiber that is under continuous tension, then the fracture stress will be reached at a strain of about 2%, putting the stiffness at about 25 GPa. Although far higher than the stiffness of collagens so far measured, this value seems much more in line with the stiffness to be expected of a covalently bonded fiber. Interestingly, using a rheological approach, Amis et al. (1985) found the stiffness of the helical part of type I collagen in solution to be five times larger than that of coiled-coil α -helical protein. If that value is 6–10 GPa (the highest estimate from measurements of mammalian keratins), then that puts the collagen triple helix at 30–50 GPa. To some extent collagen can be compared with silk and feather keratin in that covalent bonds take the main stresses, and hydrogen bonds have to cope only with shear, so that sounds much more reasonable. Both these estimates are about 10 times the “normal” measured stiffness of tendon collagen in the steeply rising part of the curve, which suggests that this shortfall is a result of the relatively low energy bonding between the various components in the hierarchical structure of tendon.

Collagen in tendon is used in two main ways: at high and medium strain rates and with static loads. In both these modes of use it is unlikely that the collagen is ever totally unloaded. It is much more likely that even in the tendons of a running animal the strains are such that the material spends most of its time working in the high modulus region, even when the associated muscles are not contracting. This is even more obviously true for collagen in arteries that are, as simple dissection will show, in a state of prestress. Apart from maintaining the collagen in its high modulus region, the importance of such prestress is obscure, even though it seems to occur in many tissues other than arteries. At the high strain rates that occur during running and walking it is likely that the modulus is higher and that viscous loss processes are less important, as there is simply not enough time for them to occur. It is also probable that the toe region is less evident. At lower strain rates with a constant rate of extension, conditions

under which most tests are performed on collagenous tissues, the modulus is much lower, and so extensibility up to the ultimate stress is greater. It is therefore probably dangerous to extrapolate results from medium-strain-rate experiments to situations in which the strain rates are much higher. Under sinusoidal straining at rates varying from 0.2 to 11 Hz there is little or no dependence of modulus or energy dissipation on frequency (Bennet et al. 1986).

The whole question of how collagen functions in the living animal is a very vexed one. Tendon withstands static loads in the Achilles tendon of a standing cow or man. Tendon does stress-relax, but within the limits of reversible stress-strain behavior, it performs very similarly to bulk crystallized polyethylene. This observation confirms the crystalline nature of collagen, since such a broad spectrum of relaxation times is typical (though not necessarily diagnostic) of a crystalline material. At a strain of 0.075 the tensile behavior is no longer reversible and results in breakdown of the tendon at long times. It might be thought that, since rat tail tendon is not normally statically loaded for long times, Achilles tendon and other such collagenous structures would be more tightly cross-linked so that they could achieve an equilibrium modulus of useful value.

However, it is as a reinforcing feltwork that the action of collagen is least understood. Some of this lack of understanding has to do with the philosophy of mechanical testing and the fact that biological materials are much softer than the materials for which most mechanical tests have been devised. The collagen fibers in human (for that matter, mammalian) skin are oriented like a felt, so that mainly only a limited net orientation of the collagen is expressed as local differences in extensibility. Whereas it is obviously adaptive for the skin over the lower abdomen to be able to stretch in all directions, it is not so obvious that skin elsewhere can be more adaptive if it stretches less in one direction than another. This property was investigated by Langer, a person of much tact and discretion, who mapped the extensibility of skin over the entire human body. He summarized his finding as lines (now known as *Langer's lines*) drawn on the skin that indicate the direction and relative magnitude of extensibility (Gibson, Stark, and Kenedi 1971). These lines, we now know, are closely related to the local orientation of collagen in the dermis.

Apart from the satisfaction of innate curiosity, other reasons for wanting to understand the basis of the stress-strain characteristics of skin are both medical (plastic surgery, design of prostheses, replacement skin for burn victims, etc.) and engineering. Because such soft tissues are remarkably widespread in the animal world and so may represent a useful, safe, and efficient way of containing materials, especially under pressure, they could be modeled if we knew the relevant characteristics. Jim Gordon once calculated that when one yawns, the strain energy density in the skin covering the cheek is about the same as that in a piece of mild steel just before fracture!

2.3.4 PROTEIN RUBBERS

The proteins discussed so far have been fibrous with a regular periodicity along the peptide chain that forms regular structures. The major bonds within these fibers are oriented along the main axis of the fiber; consequently, these materials have a

relatively high modulus and low extensibility, which is typical of a crystalline material. What strain they can accommodate in excess of, say, 0.05 is due to the presence of regions where the protein chain has greater mobility. The source of that mobility can perhaps be understood after one has examined some of the proteins that have evolved in response to the need for the elastic storage of strain energy at low stiffness. These materials are more like the rubber that was discussed briefly in section 1.3. The most studied of these are resilin, abductin, and elastin. Their commonality is that entropic elasticity requires space, but is the space, and the way it is used, supplied in the same way with all these materials? An excellent overview of this area is provided by John Gosline (Gosline et al. 2002)

2.3.4.1 ELASTIN

Elastin is the main elastic protein of vertebrates and is usually found in association with collagen. It is very stable when heated and is almost defined as that fraction remaining after collagen and other components have been removed by autoclave treatment at 110°C. Elastin is most commonly prepared from the yellow neck ligament (ligamentum nuchae) of ungulates, which counterbalances the weight of the head in the same way that some “up-and-over” garage doors are counterbalanced by large springs and considerably reduces the muscular effort required to move the head. The ligamentum nuchae is about 80% elastin and has a shear modulus of around 0.6 MPa. Elastin is covalently cross-linked by isodesmosine, desmosine, and lysinonorleucine, three unique amino acids. As a result of this cross-linking, elastin is rendered largely insoluble and can sustain loads.

There has been considerable discussion as to the nature and origin of the elasticity of elastin (Gosline 1980; Urry 1983). Two of the rival theories, now apparently defunct, required elastin to have an entirely random structure, an attribute supported by two lines of evidence: NMR studies show that (at least some of) the chains are kinetically free and therefore not involved in any fixed structures. The lack of fixed structure was confirmed by X-ray diffraction, which showed no spots that would indicate short-range structure and which also showed that on extension the protein does not become appreciably oriented or crystalline, suggesting that it remains essentially random. These observations, together with low modulus and high extensibility (though only about 15% of the extensibility of rubber), suggested that elastin is rubbery and that its mechanics can be explained quite adequately by rubber elasticity theory (Hoeve and Flory 1974). However, the amino acids of elastin are largely (60%) nonpolar, and only 5% are polar. This composition led to the proposal that in an aqueous environment the conformation was globular, rather like droplets of oil in suspension (Weis-Fogh and Andersen 1970). This interpretation was supported by several observations: in less polar solvents, such as mixtures of formamide and water, the stiffness drops as the stabilizing hydrophobic forces in the “oily” center of the globule are disrupted, and the protein chains gain greater freedom of movement. Also, a fluorescent probe—a molecule that changes its fluorescence according to the polarity of the surrounding medium—can be bound into the hydrophobic region of the elastin molecule. Changes in the fluorescence of the probe as the elastin is extended show that extension is accompanied by an increase in hydration of the

hydrophobic region, a conclusion supported by thermodynamic data (Gosline 1980). However, sections of repeating sequences of peptides—the pentamer (VPGVG) and the hexamer (APGVGV)—and the observation in the electron microscope of fibers in preparations of dispersed elastin suggested that randomness was not pervasive (Urry 1983), which in turn makes it unlikely that elastin is totally rubbery and random. This was the position adopted by Urry.

His main problem was to reconcile the apparently strong patterns indicated by the presence of fibers and molecular structure with the data suggesting rubbery elastic mechanisms. He did this by proposing a coiled-coil structure. Such a structure will form fibers yet will not give a well-defined X-ray diffraction spectrum because the structure is so open and “long range.” Water interacts with the polar amino acids but not with the hydrophobic ones. Instead, it forms internally bonded structures (clathrate cages) that sit between the hydrophobic areas of the protein and stop the protein chains from interacting, force them apart, and lead to an open helical structure with the clathrates down the middle. Such cages occur in water sitting on a hydrophobic surface, which give the water a very high viscosity (Liu, Zhan, and Lua 2007; Zhu and Granick 2001), so the water is in a rather strange condition. The amino acid sequences give rise to β -turns (figure 2.22) that produce an open helical structure (figure 2.23) arranged around a core of clathrates and that form each of the three

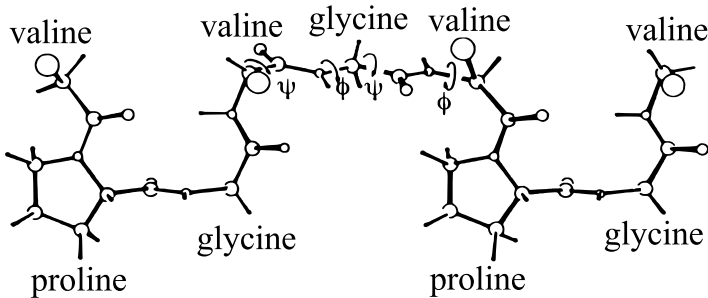
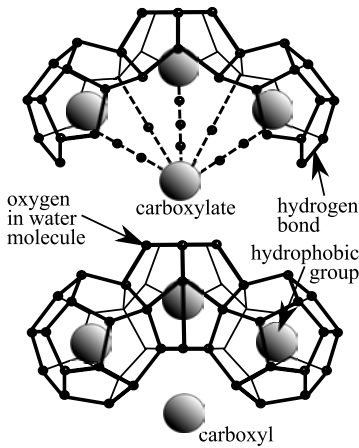


Figure 2.22. β -Turn of elastin, showing the rotations that lead to the elastic properties (Urry 1983).



Figure 2.23. Assembly of β -turns into the primary elastic helix, which is further assembled into fibers (Urry 1983).

Water destructured by charge



Water in its structured state

Figure 2.24. Effect of charged and uncharged groups on the structure of surrounding water.

strands of the coiled coil. Clathrates are structures of water (figure 2.24) and thus belie the concept of water as composed of randomly interacting molecules.

The reality of this hypothesis is supported by a large amount of chemical and physical analysis and modeling. According to Urry, the elastic properties reside primarily in the rotational freedom of the bonds in the β -turn (indicated in figure 2.22), the so-called librational entropy mechanism. This freedom of rotation is assured by the hydrophobic nature of the protein and the large amount of water entrained. It also explains why elastin was originally thought to be rubbery: the β -turns are so flexible that they can adopt a wide variety of shapes and so can be modeled as entropic—which they are, but they represent only a small part of the molecule. However, it is possible that the exchange of entropy between water and the elastin has some bearing on the elastic response. A computer model of an elastin-like peptide, (VPGVG)₁₈, was cyclically deformed at 10°C and 42°C at high speed, which was consistent with experimentally determined dielectric and NMR relaxation time scales. The simulations suggested that the entropy of the water associated with the hydrophobic groups decreases during extension and increases on relaxation. The mobility of the main chain appears greater in the extended than in the relaxed state, which suggests that hydrophobic hydration is an important factor in entropy-based elasticity (Li et al. 2001).

However, at temperatures higher than ambient, but depending on the chemistry of the elastin and its degree of hydrophobicity, the clathrates break down, the water becomes less structured, and the protein chains interact more closely and assume a more tightly folded shape. Thus as the temperature increases, the water gains entropy faster than the elastin loses it as it shrinks, so the total entropy increases. The change in shape of the protein is fairly abrupt and so can be classed as a transition. The shape change can be used to perform work, so that elastin can be used as a molecular

machine (Urry 1995). Similar tricks can be played with collagen (Steinberg, Oplatka, and Katchalsky 1966).

The elastic mechanism of elastin has implications in disease: One of the factors in arterial disease is the deposition of fatty deposits (plaques) on an inner artery wall. If these fats enter the elastin molecule, they can disrupt the normal elastic mechanism of elastin and make the material less resilient. This loss of resilience would make the elastin more susceptible to fatigue fracture, since it would have to dissipate more strain energy. Certainly, the elastin breaks down in diseased areas, and the change in mechanical properties of the artery can lead to lower overall fracture energy and so to greater danger of catastrophic failure of the artery wall.

2.3.4.2 RESILIN AND ABDUCTIN

Resilin occurs in insects and other arthropods, where it serves as a store for strain energy (Jensen and Weis-Fogh 1962). For instance, in the flight mechanism of the locust, resilin in the wing hinge is deflected at the extremes of the wing strokes, slowing the wing down and storing the kinetic energy of the wings as strain energy. The stored energy is then delivered back to the wing for the start of the next stroke where, as kinetic energy, it enables the wing to accelerate more quickly into the next stroke. Resilin is also used to store energy delivered to it at a relatively slow rate by muscular contraction, delivering it at a far faster rate (power amplification), thus enabling the flea (among other insects) to jump (Bennet-Clark and Lucey 1967) and the mantis shrimp to make its rapid strike (Zack, Claverie, and Patek 2009). Abductin occurs as the inner hinge ligament of bivalves, where it acts as the (passive) antagonist to the shell adductor muscles (Kelly and Rice 1966). It functions as a simple rubber pad in compression.

Resilin and abductin are typified by high amounts of glycine—more than 55% in abductin from some molluscs and 15% methionine, where amino acid sequences such as NAGGFGGIGG, GGGPGGFGGIG, GGSGFGG, and GGGLGGFGGI are typical in the main part of the protein (Cao, Wang, and Bayley 1997). In resilin from *Drosophila melanogaster*, the fruit fly, there are 18 repeats of SDTYGAPGGNGGRP and 11 repeats of GYSGGRPGGQDLG (Tamburro et al. 2010) showing high proline and glycine content. Both proteins are strongly charged and so are not playing elastin's tricks with the plasticizing water. Glycine allows the most steric freedom in a protein chain, and proline inhibits formation of regular structures (Nairn et al. 2008). Both elastomers are able to recover completely after applied deformation, implying that the cross-links (tyrosine derivatives in both materials) are covalent (Andersen and Weis-Fogh 1964; Andersen 2004).

We seem to be set up for truly rubbery materials. How do we demonstrate this best? It seems that the least contentious method is to show that the elastic restoring force is provided mainly by entropic means, which requires measurement of mechanical properties at a range of temperatures. Because the proteins are so polar, one would not expect them to precipitate (strictly, to coacervate, or to form a hydrophobic droplet), owing to reorganization of the water with increasing temperature. However, problems remain, since one of the requirements of the thermodynamic approach is that the volume of the specimen remain constant. With the possibility that water will

move in and out of the specimen as the kinetic activity of the polymer chains varies, this is a very real problem. It's overcome by making sure that temperature and volume are at equilibrium before a reading is taken. The sample is then deformed by a given amount and that deformation retained while the temperature is changed slowly to allow the swollen volume to maintain its equilibrium. The formulation is then

$$F \approx \frac{\partial S}{\partial L_{T,V,eq}} + T \frac{\partial F}{\partial T_{\lambda,P,eq}}, \quad [\text{Eq. 2.1}]$$

where F is force, S is entropy, L is length, and T is temperature; the subscripts, implying factors to be held constant, are V (volume), P (pressure), and λ (compression ratio, or deformation); and eq indicates equilibrium conditions. The entropic contribution to the measured elastic restoring force is the slope of the line of force plotted against temperature. The contribution of internal energy is the calculated force at 0 K, which is zero for an ideal entropic (rubbery) elastomer. With both resilin (Weis-Fogh 1961a, 1961b) and abductin (Denny and Miller 2006) the outcome (figures 2.25 and 2.26) is that the materials are rubbery. This is a bit of a surprise for those of us brought up on the idea that all molecular information implies structure, and that patterned information implies patterned structure.

However, not all the energy expended in deformation (measured as the area under the stress-strain curve on extension) is recoverable (measured as the area under the

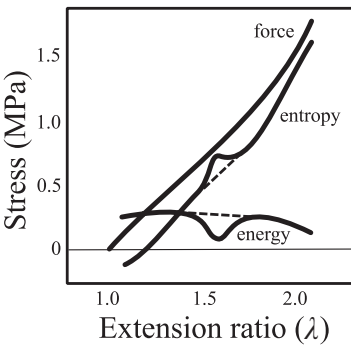


Figure 2.25. Contributions of internal energy and entropy to the elasticity of resilin (Weis-Fogh 1961).

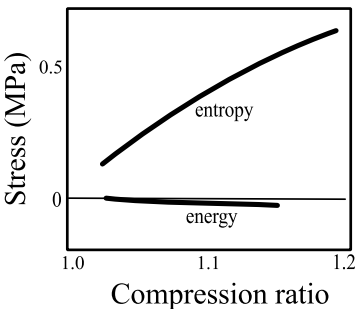


Figure 2.26. Contributions of internal energy and entropy to the elasticity of abductin (Denny and Miller 2006).

stress–strain curve on retraction to zero stress and strain). The ratio of energy recovered to energy input is the resilience, $R = 1 - 2h$, where $h = \pi \tan \delta$ and is the elastic loss factor. The function $2h$ is also equal to the area of the hysteresis loop, which is analogous to the stress–strain loop derived in dynamic tests, but note that the hysteresis loop is obtained from a straight-line ramp function. Dynamic tests are conducted with sinusoidal straining (section 1.3.3). R , then, is a measure of the efficiency of the rubber. Resilin has a resilience similar to that of elastin, about 98%. The variety of abductins is much greater. Swimming bivalves that open and close the shell several times a second require an efficient hinge ligament, and its resilience is as good as that of resilin. Sessile and digging bivalves do not move their shells so often or so fast, and this difference in behavior is correlated with lower resilience of the hinge ligament, nearer 80% (Kahler, Fisher, and Sass 1976). This value is probably at least partly related to variations in the amount of glycine present and to the amount of embedded aragonite in the hinge (Ono et al. 1990; Timmermann 2003).

2.4 Coping with Strain Energy

Collagen, elastin, resilin, and abductin all function as stores of strain energy for use during locomotion but are by no means the only materials to do so. To be of any use as an energy store, these materials have to be resilient. But this will make them brittle, since if a material has no way of absorbing energy, then that energy can be used in crack propagation and the creation of new surfaces (section 1.4). Insect cuticle seems to be a good example of this type of system. For instance, the apodeme of the hind leg of the locust forms a major part of the energy storage system for its jump (Bennet-Clark 1975). Because the tendon is so stiff (section 5.2), it stores energy at relatively low strains—about 0.03—but can store twice the amount of energy that resilin can, since it is 200 times stronger than resilin and works at 1% of the strain at which resilin works. The tendon is loaded to within 15% of its ultimate strength before a jump, but this is quite safe (mostly!), since the tendon is loaded relatively slowly. As a rough estimate, the size of crack that a piece of resilin could accommodate without fracturing under load would be, at 0.25 mm, rather larger than that which a locust apodeme could accommodate under comparable conditions (i.e., 0.1 mm) for a similar amount of energy stored. These measurements are not necessarily smaller than the size of the pieces of cuticle in question, so resilin pads are at risk of catastrophic failure only in larger insects. This situation is not too bad if the elastic energy stores are kept out of harm's way, as is the tendon down the middle of the hind leg of the locust. Even so, it is difficult to load the apodeme experimentally to a value approaching its natural strength, and this difficulty has been attributed to surface cracks initiated during the preparation of the specimen (Bennet-Clark 1975).

By contrast, the collagenous tendon of vertebrates is about a sixth as strong as locust tendon and a tenth as stiff. But because it can work at higher strains, it can also store larger amounts of strain energy. Both collagenous and cuticular tendons have to work at low strains, since they are in series with muscles whose efficiency will drop off if they have to contract too far: the locust tendon probably needs to be

stiffer because the muscle is more rigidly contained by the exoskeleton and so is not able to change shape to any great degree. Collagenous tendons differ in another way from the other energy stores mentioned so far: they are fibrillar. This difference is important, since although the tendon can have resilience of 90%–94% (Bennett et al. 1986), at strains above 0.04 the tendon can absorb energy by separating fibers laterally (defibrillation) within the bundles. The resulting damage can be assessed and related quantitatively to the amount of energy absorbed. In addition, if the matrix is relatively compliant, it will not transfer shear stress so readily from one fiber bundle to the next, so that the normal mechanisms of crack propagation will be frustrated (much the same sort of thing happens in grass leaves [section 5.2.3] and in rope). Thus the tendon can absorb energy in a manner that does not affect the longitudinal continuity of the fibers and hence the load-bearing capacity of the tendon. At higher loads, the fibers start to break and pull out, absorbing even larger amounts of energy. Thus the fibrillar architecture of tendon gives a highly resilient yet very tough energy store—a great advance over resilin, though not so dissimilar from locust leg tendon.

One can understand the difference between these energy storage materials when one considers their morphology in use. Resilin and abductin are nearly always used in compression or bending and are rarely loaded in tension. Thus the low toughness is not a disadvantage, because the material is rarely exposed to conditions favorable to crack propagation. But the dragonfly has a pure resilin tendon that is loaded in tension as part of the flight system (Jensen and Weis-Fogh 1962). Its margin of safety has not been calculated, although, like the locust apodeme, it works within the insect and so is unlikely to be exposed to scratches that might initiate cracks. The other unusual thing about such use of resilin is that it is a high-strain rubber being used in series with a muscle—superficially, a Bad Thing (see earlier discussion). Presumably, the wing muscle of the dragonfly is attuned to the resilin in some way, but this relationship has not been investigated.

In contrast with most resilin and abductin systems, the leg tendons of a running sheep or man are loaded in tension and at a high strain rate, possibly even equivalent to impact loading in a sprinter. Under these circumstances the possibility of overloading is much greater than in the locust, where the tendon is loaded in a relatively slow and controlled manner, although such tendons are rather subtly matched to their working loads and strains (Ker, Alexander, and Bennett 1988). The human Achilles tendon can be fractured if the ball of the foot is loaded very quickly, as in a sprint start off blocks or a stumble down stairs. A subcritical load can cause excruciating pain. As a corollary, the abductins of nonswimming bivalves are much less resilient, which may indicate that the hinge ligament is being selected for toughness in holding the two shells together. The resilience of tendon seems to be independent of temperature between 20°C and 36°C (the range measured so far). Other elastomers deal with lower temperatures differently. The abductin of a cold-water scallop (*Aequipecten colbecki*) maintains a higher resilience at low temperatures than does the abductin of a temperate-water scallop (Denny and Miller 2006). It appears to do this by raising the glycine content to more than 70%!

Although some protein materials store elastic strain energy and use it at a different time or more quickly than the energy can be delivered by a muscle, a number of

materials have evolved to dissipate energy (Gosline et al. 2002). The silk of the spider's web is a good example, as is the byssus thread of *Mytilus*, the common mussel, and related bivalve molluscs (Pearce and LaBarbera 2009). The byssus threads guy the mussel down, resisting wave action that can induce drag forces equivalent to a wind of 1000 mph (1 km in a little over 2 s). This is an ideal biological material to investigate, since it is conceptually so simple—a tensile cord—and has a well-defined function—to hold the mussel onto the substrate. Functionally, it is much more complex. The adhesive disk has to stick strongly to a variety of materials (mostly the bacterial film that covers most marine surfaces) and transfer force smoothly from the thread. The thread has to transmit forces generated by drag as waves pass over the mussel, minimizing the shock by spreading it out over time. It therefore has to be strong and stiff but capable of storing significant amounts of strain energy. Finally, this stiff structure (or a number of them) has to be rooted firmly in the soft body of the mussel. Depending on its degree of exposure to wave action, the mussel is held on by 15 to 60 of these threads. The threads are formed by a complex of glands in the foot, which manipulate (pedipulate?) and mold their secretions. Since the threads are 2 to 4 cm long, they represent small but readily manageable specimens whose mechanical properties can be relatively easily measured and related to the ecology of their owner.

At the molecular level, the threads are remarkably complex, having protein components related to collagen, elastin, abductin, and silk (Harrington and Waite 2007) that vary from species to species, apparently related to the mechanical environment to which the animal is regularly exposed. The basic protein throughout is a fibrous form of collagen with built-in kink. Within the collagen molecules are domains that resemble the other three proteins, so that each collagen molecule is like a block copolymer with varying stiffness and extensibility along its length. What this mixture means in terms of mechanical properties is indicated by conveniently comparing the stiffnesses of the components (figure 2.27) on a log–log scale (Gosline et al. 2002). The three types of collagen are distributed differently along the thread (figure 2.28). The collagen that has the equivalent properties of abductin (identified simply by its high glycine content) is distributed along the entire length of the thread, but the extensible proximal section has a predominance of elastin-like motif, and the stiffer distal part has insertions similar to silk fibroin. Meanwhile, the plaque sticks to the rock by means of a much-studied high-DOPA polypeptide (Lee, Scherer, and

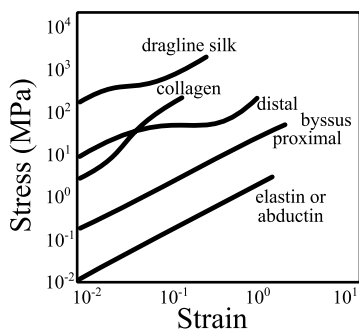


Figure 2.27. Comparison of fibrous proteins in animals (Gosline et al. 2002).

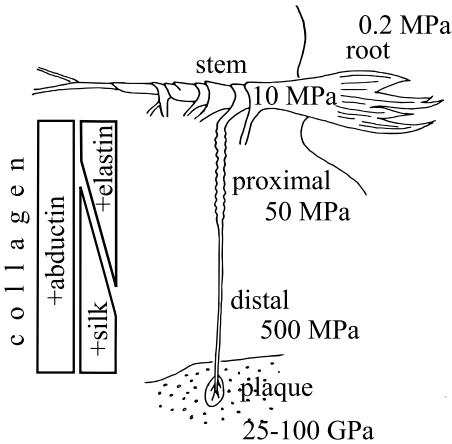


Figure 2.28. Compliance matching by the byssus of a mussel.

Messersmith 2006) that is nonselectively adhesive partly by hydrogen bonding and partly by covalent bonds, in a manner still not understood. The overall effect is to provide functionally graded mechanical matching of the mussel to the rock, more or less getting rid of stress concentrations that could initiate fracture (Waite et al. 2004). And the stretchy proximal part of the thread acts as a shock absorber. Remarkably, not only is the entire thread covered with a waterproof varnish (probably DOPA-based) but it is, of course, secreted as a highly hydrated material that is rendered insoluble within a few minutes, probably involving phosphoserine. Most remarkably, if the mussel doesn't like its current position, it can discard the byssus and move to a better place, guying itself in position again!

Sugars and Fillers

At the outset it should be realized that, relatively speaking, less is known about the polysaccharides than about the proteins. This is largely a chemical problem—amino acids have much more convenient handles for chemical processing and identification, which also makes them more powerful chemically. They are also more intimately associated with the genetic code, which has persuaded biochemists and biologists that protein structure is more important and basic. It is also much easier to deduce the sequence of a protein from the chemistry of the gene. Ironically, it is the sheer chemical anonymity, combined with extreme abundance, of polysaccharides in general that makes them commercially far more significant—as cellulose and chitin (natural and processed), carrageenan, agar, and the like. Such products are used for building, making paper, stabilizing food substances, making fibers of all kinds for clothes and ropes, and in the dyeing industry. As general references, use Rees (1977) and Walter (1998). Additionally, the gel-forming abilities of many polysaccharides rely on both their ability to bind water and, crucially, on the strange structural properties of water, which are still being explored (Zheng and Pollack 2003).

The polysaccharides that have any mechanical significance are made of hexoses (figure 3.1a), which can occur in any one of four main conformations (figure 3.1b). With glucose (β -D-glucopyranose) the commonest or most likely conformation is the 4C_1 chair, since this has the lowest internal energy. Its bonds are least strained in this conformation, and the various groups sticking out of the ring interfere with each other to the least extent. The sugar shown is the β -form with the OH on C-1 above the plane of the ring. In the α -form the OH and H on this carbon are reversed. The D in the name means that the molecule rotates the plane of polarization of incident light dextrally, or to the right, when it is in solution. The levorotatory, or L form, of the pyranose sugars is rarer in biological materials, but it does occur, for instance, in the pectins that stick plant cells together, which contain L-rhamnopyranose (rhamnose). The chemical properties of the basic sugar unit can be changed by changing the side groups. These changes are reflected in the different names given to the resulting sugars. For example, if the OH on C-2 is replaced with the group —NHCOCH_3 , the result is β -D-N-acetylglucosamine; if the H_2OH on C-6 is replaced with $\text{H}_2\text{SO}_3\text{H—}$, the result is the acid sulfate. These molecules and more are shown in figure 3.2. In this way it is possible to produce sugar units with a variety of charge, chemical, and steric properties.

Another variable is the linkages between the sugar units. Amino acids are linked only through the peptide link, which has fairly consistent steric properties. Sugars can be bonded, by a condensation reaction, by any of the OH groups in the molecule. In

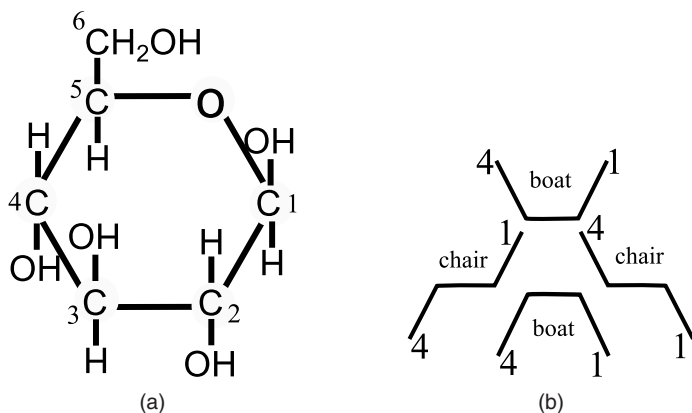


Figure 3.1 (a, b). Haworth formula and "boat" and "chair" conformations of hexose.

pyranose there are five of these, on C-1, C-2, C-3, C-4, and C-6, and each of these can exist in α or β form. Thus there are 10 possible bonding sites and 100 ways in which two pyranose molecules can link to form a disaccharide. In practice, this number is much smaller, but it does suggest that there must be very strict control over the way the monomer units are assembled. The bonds are named according to whether they are α or β and by their position on the sugar ring. Thus the cellobiose (bi- = two sugars) molecule of figure 3.3 is made of two β -D-glucopyranose units joined by a β -1,4 linkage. Just as with the peptide bond, there is freedom of rotation about the linkages joining the sugar rings, so a conformation map can be drawn in a very similar manner to a Ramachandran plot (section 2.2). The two angles are again called ϕ and φ . Figure 3.4 shows the conformation map for cellobiose. The point X corresponds to the conformation found experimentally by X-ray diffraction in crystals of β -cellobiose, C marks the cellulose conformation, and T marks the theoretically most probable (lowest energy) conformation. There is no experimental evidence that the β -1,4 linkage ever exists in any significant amount in the smaller lower zone (unmarked), either in the solid state or in solution. The linkage is further stabilized by hydrogen bonding. Such bonds can occur between any O and H, so there is a far greater range of stabilized conformations than with proteins, where the H-bonding possibilities are much more limited. Figure 3.5 shows the H-bond that normally occurs with a β -1,4 linkage. Obviously, this sort of conformation is more stable in the crystalline state: in an aqueous environment the water molecules compete for the H-bonding sites, and the rotation about the two bonds joining the sugar rings is much greater, since the H-bonds between the rings are less permanent. There is therefore a tendency toward a random coil formation. However, as with proteins, there are many types of conformation that are extensively stabilized by H-bonds and that have been detected in crystals and characterized by X-ray diffraction. They have been shown, in several instances, to occur in solution as well. The reason is the same as with proteins: a regular structure such as a helix that is stabilized by H-bonds and other forces becomes more stable as it increases in size and cooperative interactions become more significant.

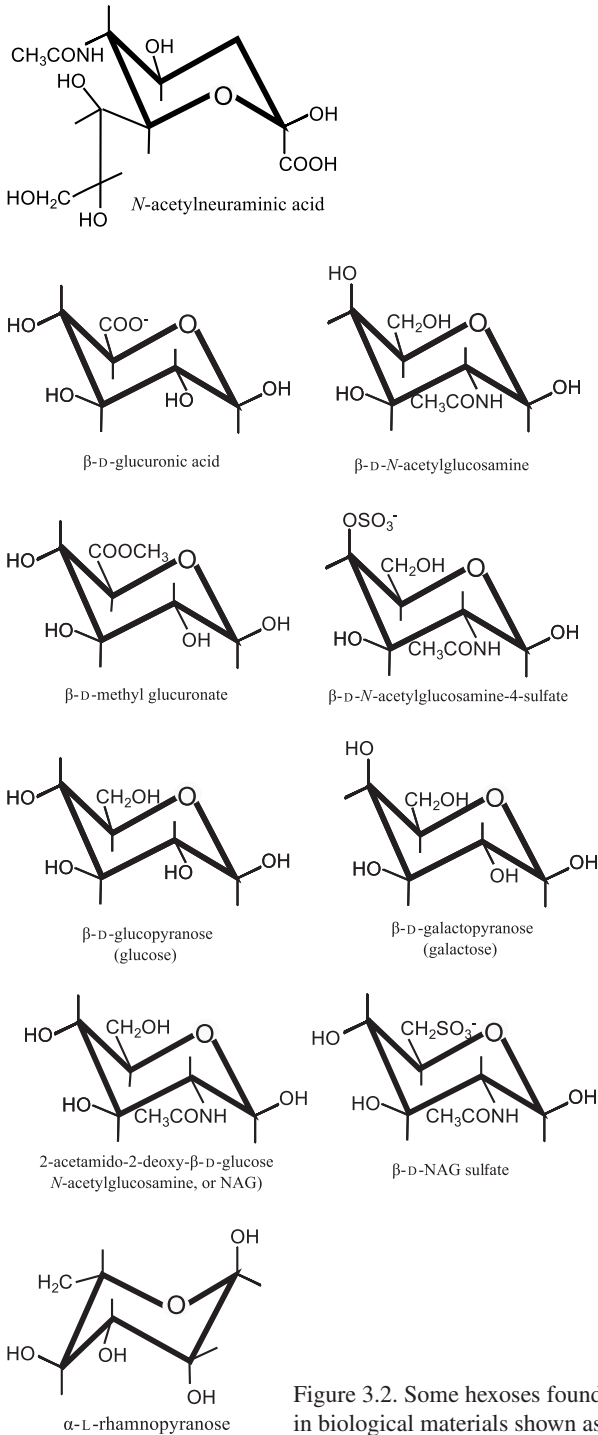


Figure 3.2. Some hexoses found in polysaccharides occurring in biological materials shown as conventional formulas.

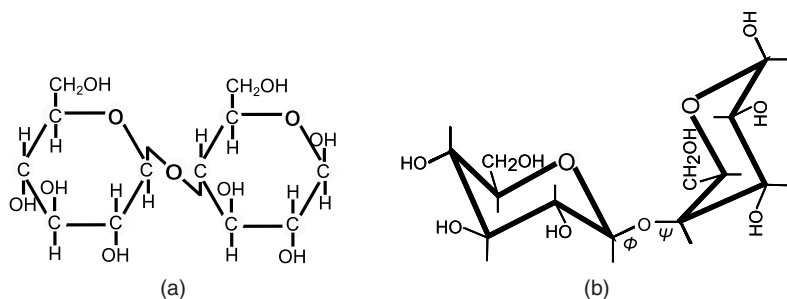


Figure 3.3. (a) Structure of cellobiose shown as Haworth formula, (b) the same, showing linkages each side of the linking O about which rotations occur.

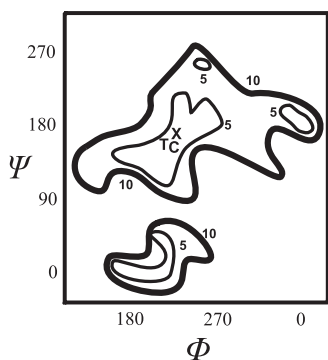


Figure 3.4. Conformational energy map for cellulose (contours correspond to energy levels of 5 and 10 kcal mol⁻¹). T = lowest energy state; C = actual state of cellulose; X = actual state of cellobiose. (Rees 1977).

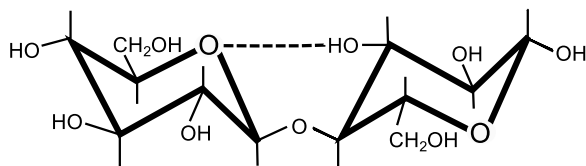


Figure 3.5. Hydrogen bonding associated with the β -1,4 linkage in cellobiose.

Linkages between any of the ring carbons have only two bonds about which to rotate. But linkages involving C-6 on the side chain will obviously be much more mobile, not only because there are three bonds about which the units can rotate but because the two rings are farther apart and are thus less likely to interfere with each other. In structural biopolymers this linkage is most common at the junction of polysaccharide chains to protein chains, which seems mostly to occur at serine and asparagine residues. The linkages define the conformation of the polysaccharide. For a good summary of this subject, see Rees (1977). Table 3.1 summarizes the consequences of the most common linkages and the conformations produced if only one type of linkage is present.

Many structural polysaccharides contain more than one type of linkage and more than one type of residue. There are two main ways in which the residues can be arranged along the polymer: periodic (ABABABA . . .) and interrupted (AAAAA-

TABLE 3.1
Effects of sugar–sugar linkages on molecular conformation

<i>Most likely conformation</i>	<i>Geometric relationship of residues</i>	<i>Linkage</i>
Ribbon	Zigzag	β -1,3 β -1,4 α -1,3 α -1,4 (glucose, galactose)
Hollow helix	U-turn twisted	β -1,3 α -1,4 β -1,2

Source: Rees (1977)

BBBAAAABBBBBBAA . . .). In the former there is a strong pattern in the arrangement of residues; in the latter the residues are arranged in blocks of varying length. Because the types of linkages between the residues may vary, it is difficult to predict the conformation of these molecules, so a combination of computer modeling and X-ray crystallography has been resorted to and found successful. These techniques have shown that periodic chains with mixed linkages can form anything from undulating ribbons to extended hollow helices.

In summary, a comparison of sugars with amino acids as building blocks reveals two major areas of difference:

1. There is far less variety in side-chain types of polysaccharides with respect to size, conformation, and polarity/charge characteristics. There are no hydrophobic interactions. Thus high hydration and/or hydrogen bonding or ionic interactions are possible.
2. A far greater variety of bonding is available between polysaccharide residues, which means that a greater variety of periodic structures can be generated. These structures occur over lengths of chain that are sufficiently long to allow weak attractive forces to accumulate and to have the strength of a stable and essentially permanent bond when two or more chains of complementary structure come together. Three main types of biomaterial are exclusively polysaccharide, namely, fibers, elastic gels, and viscoelastic gels. To some extent, mainly under experimental conditions, these states are interconvertible. Thus it is possible to form fibers from elastic gels (e.g., carrageenan) and viscoelastic gels (e.g., hyaluronic acid), which allows their study by X-ray diffraction. It is also possible to produce gels from fibers (e.g., from chitin or cellulose), and this reaction is the basis of a number of industrial processes.

3.1 Fibers

Of the fibrous polysaccharides, the most abundant are chitin and cellulose. These two have very similar primary structures (figure 3.6). Some evidence suggests that every sixth or seventh residue of chitin is not acetylated, but apart from that difference, the two polymers are homogeneous. Both chitin and cellulose are probably polymerized

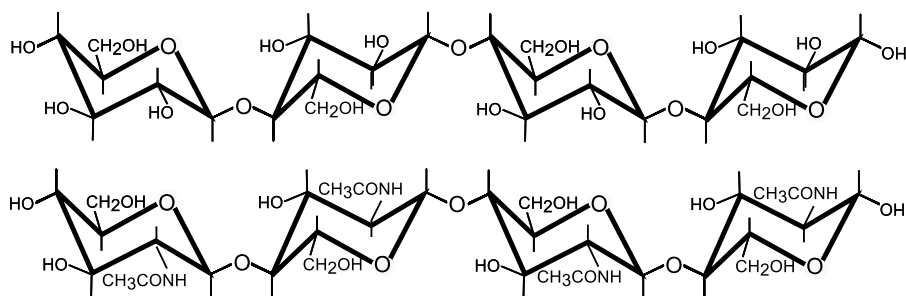


Figure 3.6. Cellulose (top) and chitin (bottom).

just outside the cell membrane, possibly “spun” off the polymerase enzyme in such a way that they can immediately polymerize into the highly hydrogen-bonded elementary fibril with H-bonds along and between the chains. As with protein β -sheets, it is possible to have parallel and antiparallel forms, though the latter is not natural for cellulose. The cellulose elementary fibril is about 3.5 nm in diameter and contains about 40 molecules across the section; that of chitin (in insects) is commonly about 3 nm and contains 19 molecules across the section. Elementary fibrils of up to 300 nm in diameter have been reported for chitin in insects, and up to 25 nm in crustacea. Cellulose elementary fibrils can be arranged into larger fibrils (microfibrils) 20–25 nm in diameter; this might be the case in crustacean chitin.

Both chitin and cellulose are stiff in tension. Micro-Raman spectroscopy (a form of infrared spectroscopy that can be performed on very small samples [Eichhorn et al. 2003]) indicates that cellulose has a modulus of about 140 GPa. A slightly higher value for cellulose has been obtained by calculation from the crystal structure, taking into account straightening of the covalent bonds and stretching of interchain H-bonds. If the H-bonds are ignored, the calculated modulus drops by a factor of 8 or so, which shows that the H-bonds are making a significant contribution to the stiffness. Chitin would be expected to have a higher stiffness still, probably about 180 GPa, since the acetyl group not only provides more H-bonding but reduces the flexibility of the linkage due to steric hindrance (Minke and Blackwell 1978). The stiffness of bulk cellulose and chitin will be lower than these values, since the degree of crystallinity is rarely perfect, and the orientation of the fibers is not strictly parallel. Regenerated cellulose can be useful here, since it can be produced in amounts large enough to put in a standard mechanical testing machine. The degree of orientation of the cellulose molecules can be varied in the same way as can spider silk—by stretching it. The greater the initial stretch (draw ratio), the more aligned the molecules are, which results in higher birefringence (figure 3.7a) and greater stiffness combined with less plastic deformation (figure 3.7b). Thus it is no surprise that the longitudinal stiffness of the plant cell increases as the orientation of the cellulose fibers more nearly approaches the longitudinal axis (figure 3.8) (Burgert and Fratzl 2009). Indeed, stretching the cell causes greater orientation of the cellulose fibers in the cell wall but also causes the cell to get thinner as it becomes longer. In dry cellulose the chains are held

together so effectively by hydrogen bonding that failure of cellulose fibers is due not to slippage of chains past each other but to breakage of the main chains (Mark 1967). The strength of a cellulose microfiber increases with the number of residues in the molecular chains, up to about 2500. Below this length, failure is presumably due to slippage of chains. Because natural cellulose molecules are three to four times longer than this, the H-bonds are, cooperatively, strong enough. This implied length criterion is probably also true for chitin, though the highest estimate of the chain length is only about 700 residues (Ker 1977). As might be expected, the stress-relaxation behavior of dry cellulose is typical of a crystalline material (figure 3.9) in that it has a

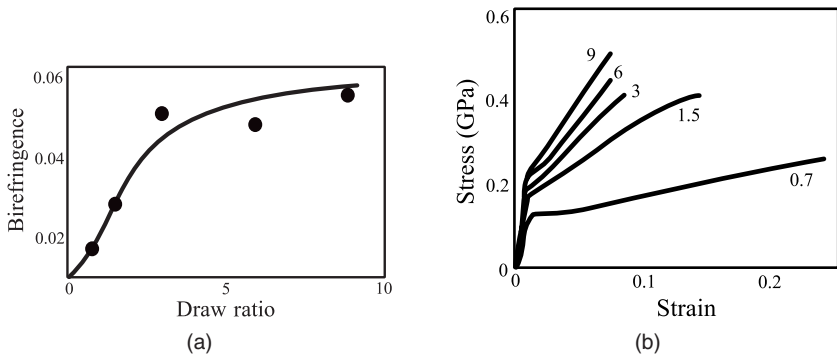


Figure 3.7. (a) Increasing orientation of molecules with draw ratio (Eichhorn et al 2003). (b) Increase in stiffness with increase in orientation (Eichhorn et al 2003).

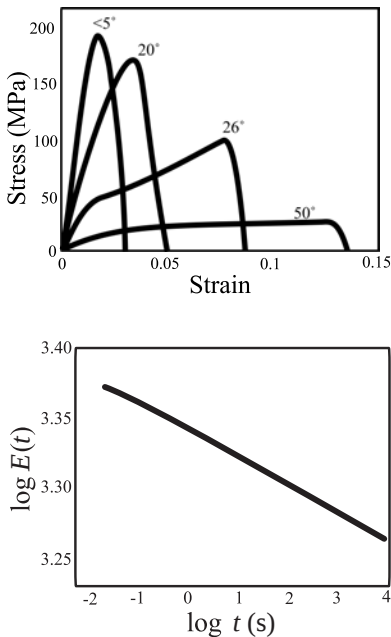


Figure 3.8. Longitudinal stiffness of the plant cell wall with the orientation of cellulose relative to the longitudinal axis of the cell (Burgert and Fratzl, 2009).

Figure 3.9. Stress relaxation of *Nitella* cell wall (Haughton et al. 1968).

very broad relaxation spectrum (Haughton, Sellen, and Preston 1968). Consequently, the modulus and hysteresis of the material are virtually independent of strain rate. This independence is truer for cellulose, since its rate of relaxation is much slower than that of polyethylene or collagen because of its extended chain conformation and the high degree of hydrogen bonding.

3.2 Structural Polysaccharides in Plants

Within the cell walls of plants, the microfibrils are cross-linked and stabilized by shorter molecules (hemicellulose) that combine the cellulose microfibrils into a network. Hemicelluloses are bonded noncovalently onto the cellulose, so that for the forces to be transmitted effectively, a significant length of the hemicellulose has to lie alongside the cellulose. For instance, xylans manage to get close enough by being a simple chain of xylose units, but they push away from the surface of the cellulose microfibril by having side chains that make this closeness impossible. The xylan can then bridge to another cellulose microfibril and bond to that with its other naked end.

The cellulosic network is not the only one in the cell wall. Other networks have been identified that can be considered more or less independently. Pectins form a network that can re-form independently (giving rise to the jam industry) and leave the rest of the cell wall apparently unaffected when they are removed. They have many large side chains that allow them to fill spaces between microfibrils and cells. Regions with no side chains occur at the ends of the molecules, allowing them to interact by forming complexes with calcium ions. Pectin chains can be cross-linked with the other networks and are important for sticking cells together. In ripening fruit the pectins are commonly made more hydrophilic and hence soluble; they cease to stick so well and cause the texture of the tissue to change. Another network is made of protein-polysaccharide complexes (glycoproteins) whose main component is extensin, a strange protein containing about 40% hydroxyproline (Hyp) that frequently occurs as part of the sequence -S-Hyp-Hyp-Hyp-Hyp. Proline is well known for its effect on the conformation of the protein backbone—the side group loops back on the peptide link (figure 2.2), which greatly reduces the mobility of the polymer. In addition, it is an attachment point for short polysaccharides. Although the mechanism is not understood, extensin stops cell elongation, probably by oxidative cross-linking. To what is not known—perhaps pectins?

3.2.1 DIRECTED EXPANSION AND GROWTH

The orientation of cellulose in the cell wall is controlled by the orientation of a network of microtubules (how are they oriented? controlled?) arranged on the inner surface (cortex) of the cell (Lang, Eisinger, and Green 1982). These microtubules form a guidance system for the rosettes of cellulose-producing enzymes as they “float” in the cell membrane and spin the cellulose fibers as they travel (figure 3.10) (Brett 2000). How the rosettes move is another matter: are they pushed or do they jump? The orientation of the cortical microtubules can be affected by external stimuli such

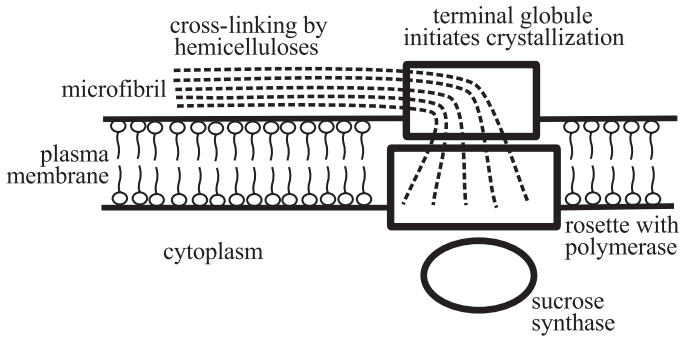


Figure 3.10. Production of cellulose in a plant cell wall (Houghton et al. 1968).

as light (amount, color), auxin (a plant hormone), and mechanical strains such as those due to bending or unequal stretching, as might be initiated by wounding (Hush, Hawes, and Overall 1990). These stimuli are additive, so a small amount of auxin makes the cells more sensitive to the other stimuli.

The shape, size, and stiffness of the nonlignified parts of a plant are governed by the mechanical properties of the thickest cell walls in the system, which, in turn, are governed by the orientation of the cellulose microfibrils and their degree of cross-linking (Verbelen et al. 2001). The degree of cross-linking is important, because if the cross-links are strong enough, they can mask the orientation effects of the cellulose. Growth is thus also controlled by the degree of cross-linking, which is, in turn, controlled by expansins, proteins that sever the cross-links and are associated with growth of plant tissues in general, and ripening and softening of fruit (Cosgrove 2000) (figure 3.11). The botanical term for this action is “loosening,” which mechanically is probably best expressed as increased creep compliance of the matrix around the cellulose fibers (see later chapters for discussions of fibrous composite materials). The softening of fruit is distinct from *mealiness*, which is the separation of cells caused by the increased solubility of the pectins that glue the cells together. In peaches mealiness is associated with reduction in extensin (Obenland, Crisosto, and Rose 2003). Interestingly, a plant also uses these processes when it gets rid of, or abscises, parts that are old, diseased, supernumerary, or injured. It has several ways of doing so (Vincent 2000). In one process, cells separate (as the pectin is dissolved)

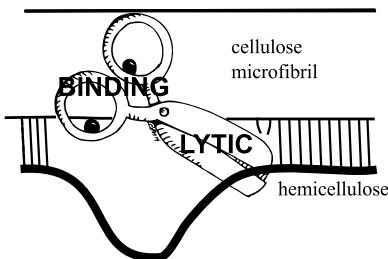


Figure 3.11. Cell-wall softening (Cosgrove 2000).

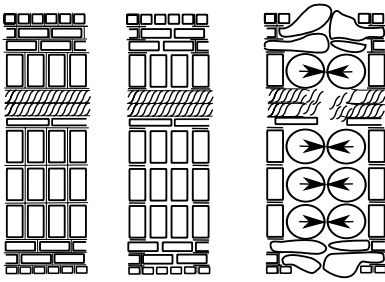


Figure 3.12. Cellular events at abscission.

and then expand under their internal pressure as the cell walls soften under the influence of extensin (figure 3.12). The rounded cells push each other away and fracture both the epidermis and the vascular tissue. The strength of fully formed vascular tissue in grass is on the order of 5 MPa (Vincent 1991). If the tissue occupies 3%–5% of the cross-sectional area (about the same as in a soft grass such as *Holcus*), then the stress developed by the bulging cells is about 0.15 to 0.25 MPa. If the osmotic pressure in the bulging cells is between 5 and 10 atm (i.e., 0.5 to 1 MPa), then sufficient force for separation is transmitted if 30% of the surface area of the opposing cells is in contact to break the vascular bundles.

3.2.2 CELLULOSE FOR SPRINGS

Plants are low-energy devices, so they tend not to move very quickly. But if they can store elastic strain energy (necessarily over a longish period), they can release it over a short period (which is power amplification, since power is the rate of doing work). Cellulose is an elastic material. It's fairly good at storing elastic strain energy, especially when it's dry, and so is responsible for shooting seeds quite long distances (Witztum and Schulgasser 1995). Cellulose isn't as good as silk, but it's still respectable and at least as good as collagen tendon (which is what we use in running). One of the most interesting examples of cellulose strain energy is its use in the Venus flytrap, *Dionaea muscipula*, which is native to the peat bogs of the North American Carolinas, which are relatively poor in nutrients. To supplement its nitrogen intake, the plant traps unwary insects by snapping the lamina of a modified leaf around them (figure 3.13). This movement can occur in a fraction of a second—far too quick to be due to a change in turgor pressure of the cells in the leaf. Charles Darwin (1875) thought that the midrib of the leaf hinged, but this is easily seen not to be the case.

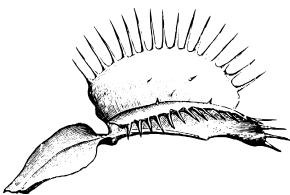


Figure 3.13. Leaf of a Venus flytrap (Darwin 1875).

Later workers thought that the turgor pressure in the cells changed or that the cell walls softened on one side of the leaf. Careful measurement of the solute content of cells before and after closing reveals no real difference, nor does measurement of the stiffness of the cell walls. The leaf reverses its curvature in closing, and this reversal is the clue. There are several examples of bent sheets of stiff material whose curvature can be reversed, and done so in the nature of a bistable. This behavior can neatly be described by catastrophe theory, a fashionable set of concepts from the early 1980s (Thom 1975). A relevant example is the slightly convex end of a soft-drink or beer can. In some examples of the species, after the end is pushed in with a satisfying “bonk,” it can spring out again under its own elasticity owing to its shape. This is an example of a shape-changing bistable that moves very quickly once triggered yet does not change its stiffness. A similar mechanism acts in the sound-producing organ (tymbal) of the cicada (Young and Bennet-Clark 1995). An even more relevant model is the anticlastic shape that a suitably prestressed sheet can produce. Not only does it flip from one stable state to another, but it reverses its curvature in the same way as does the leaf of the Venus flytrap (figure 3.14). The design is very similar—a prestressed sandwich panel (the plant uses turgor, of course). Cells in the middle layer of the flytrap are thin-walled, large, and extensible. The upper epidermis is much thicker than the lower, so that if both surfaces are in tension, the closed state is mechanically more stable than the open one. It seems likely that once the trap has closed, the changed strains in the cells are mechanically confirmed by speedy equilibration of turgor. A closed trap can be forced to open again, simply by levering the leaf laminae apart, but it takes up to a kilogram of force to do so (quite amazing for such a small and light mechanism). However, the forces controlling the shape are altered after trapping prey, and the return path to the open leaf (figure 3.15) takes longer and involves growth. This model seems very reasonable and suggests that rather than export sugars and lower the turgor pressure (and hence the powering prestrain), the flytrap softens the cell walls of the upper epidermis so that the lower

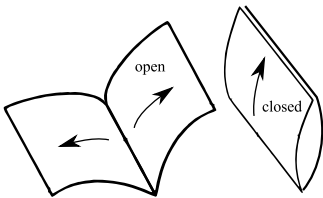


Figure 3.14. Shape changes of Venus flytrap when open and closed.

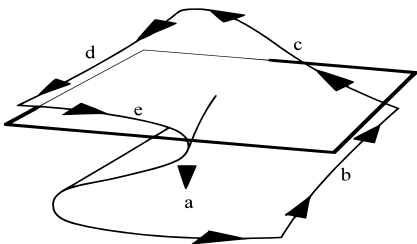


Figure 3.15. “Catastrophe” model of the opening and closing cycle of a Venus flytrap leaf.

epidermis, although thinner and therefore able to generate less force, can slowly pull the leaf open again. During this process, the upper epidermis is stretched, giving the appearance of growth. (Note that this idea has not been tested.) The open leaf can then restiffen the upper epidermis and build up its prestrain again. This model is partly supported by the observation that a newly developing leaf does not show curvature until late in its development—the two halves of the leaf are pressed together flat, suggesting that there is little prestrain in the system; whatever prestrain is present must be symmetrically distributed throughout the leaf. Only when the turgor pressure increases does the leaf curl.

3.3 Water, the Invisible Support

Water is everywhere. You can't smell it. You can't taste it. You can't really see it. Its ubiquity invites ignorance. Paradoxically we ignore it, presumably on the grounds that it's so common that it cannot be of much importance. But perhaps water is so important that it's pervasive. Which line of reasoning is correct? Water is usually thought of in chemical terms— H_2O —rather than structure. Yet it is clear from biology alone that function is derived as much, if not more, from structure, which is driven by the interaction of intrinsic chemistry and external environment. Science is well attuned to analyzing solid materials that don't move around very much or can come to a stable equilibrium under experimental conditions. We can identify molecules such as proteins and polysaccharides; we can identify their structure using X-ray diffraction and computer modeling, and this characterization is very satisfying. But with materials that are continually on the move, and are continually changing, we have problems with both experiment and credibility: with experiment because it can be difficult to measure dynamic events; with credibility because it is more difficult to ascribe causality to a system in which response to a stimulus, either known or unknown, might happen quickly and often. And with so many influences (as I sit writing this, I am in an environment where light, sound, temperature, force, and position are all in flux. And those are just the external conditions.), how many experiments, taking all variables—believable and unbelievable—into account, do we need to perform carefully to obtain reliable information? Water is also anomalous in its properties. As a gas it's one of the lightest known. As a liquid it's denser than expected. As a solid it's lighter than expected.

It's long been known that water is important to biological materials. Without it, proteins and polysaccharides would be brittle. Water molecules sit on the charged groups of the polymers and separate them, allowing them to move around and become, at the macro level, softer. Water is a plasticizer. Technically, it reduces the glass transition temperature from about 180°C to -10°C , depending on the water content. But it doesn't take long to realize that there are different fractions of water, some of which can easily be removed and some of which is quite tightly bound. In fact, some of the bound water interacts so strongly with the polymer that it can't readily be frozen: a temperature of -50°C or lower is sometimes required. This water is probably synonymous with a hydration shell or perhaps a monomolecular layer of

water that is also known as the *Langmuir layer*. With this amount of water present, the polymer has the texture of stiff leather but isn't particularly soft and certainly won't creep significantly. Adding more water, sometimes called "bulk water," increases the plasticizing effect and can give rise to a transition in stiffness that is typical of solids in which the mechanical properties are controlled primarily by hydrogen bonds, such as paper (Nissan 1976), which is made primarily of cellulose. Water does not soften cellulose fibers by a large amount—a factor of 2 to 4 as the water penetrates the amorphous regions (whence "swellulose" [Gordon, 1976])—but it greatly affects interactions between the fibers. Hydrogen bonds can be very stiff and strong—think of ice or how water is pulled up a tall tree—and they control the mechanical properties in proportion to their number per unit volume. Nissan divided the reaction of H-bonded solids into three regimes (figure 3.16). In regimes 2 and 3 stiffness is dependent on wetting; in regime 1 there is effectively no plasticization water, so tests measure stress relaxation rather than tensile strength.

Let's examine these reactions in more detail, since the interaction of water with the polar chemistry of biological surfaces, whether inside or outside the cell, is the primary driver of mechanical and morphological properties. Remember, we need to explain how water can break H-bonds nondestructively! In cellulose, consider a bond between the primary —OH of a glucose unit of one chain and the ring oxygen of another chain alongside (figure 3.17, regime 1). This bond contributes to the mechanical stability of the polymer. If a water molecule intervenes and produces regime 2, the

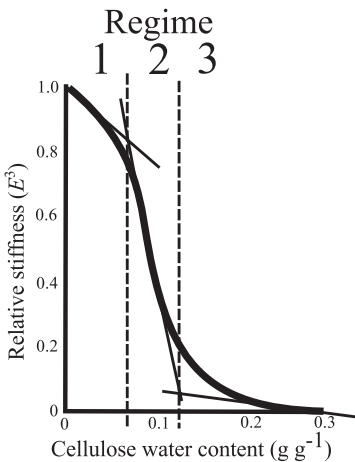


Figure 3.16. Change in stiffness with water content of a hydrogen-bonded material (Nissan 1976).

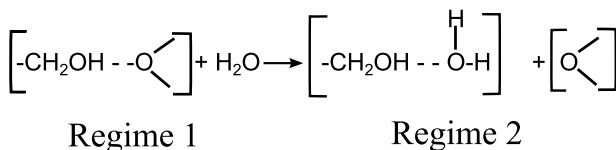


Figure 3.17. Changes in water binding with hydrogen bonding (Nissan 1976).

bond is clearly broken. The ring oxygen of the second chain can now move away from the sixth carbon on the first chain, giving both molecules more kinetic freedom and reducing the resistance of the chain to being stretched. There are now three sites with which water can interact—the two hydrogens on the newly captured water molecule and the newly liberated ring oxygen. This reaction immediately causes further spontaneous H-bond breaking in the immediate vicinity, but with a significant difference between the two regimes. In regime 1 there is insufficient water, and the interchain bond is as likely to be remade as it is to be broken. In regime 2 more water is available to be bound, and because of the greater flexibility of the cellulose molecule and competition with water molecules, interchain bonding is less likely. To quantify this situation, we use the *cooperative index*, or C.I., which is the average number of bonds broken per residue. Nissan (1976) calculates this value to be fewer than seven, which represents at least one interpolated water molecule per hydrogen on the sugar ring. However, if the cellulose molecules line up into fibrils as they are produced, each containing up to 40 molecular chains (Brett and Waldron 1996), then more water molecules must be associated with each hydrogen. The calculations are given in Nissan's paper, but for the present argument the important factors are that we have the makings of a transition mechanism with a positive feedback model and that it can be related, mathematically, to materials that are stabilized by H-bonds. Nissan proposes that such a transition is diagnostic of H-bonded materials. Standard physical-chemical methods confirm this general result—that a hydrophilic surface will attract a layer of several water molecules, and this layer constitutes a hydration shell.

However, experiment trumps this result. In recent years Gerry Pollack, a relatively unwilling iconoclast, has been rewriting the physics of water. In a series of simple experiments he showed that water assembles itself on a hydrophilic surface to distances up to 0.25 mm, representing millions of layers, not just one or two (Zheng and Pollack 2003). The observations start with the establishment of an exclusion zone next to a hydrophilic surface. The surface chosen was initially a PVA gel cast onto a microscope slide and irrigated with water containing carboxylate-coated spheres 2 μm in diameter. Within 5 minutes the spheres, initially distributed throughout the water, had disappeared from the zone at the edge of the PVA gel (figure 3.18). This

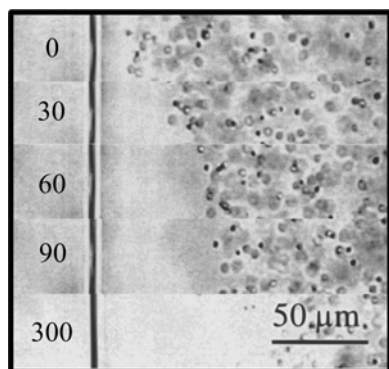


Figure 3.18. Development of an exclusion zone in water at the surface of PVA gel. The numbers on the left are time in seconds. (Modified with permission from Zheng, J.-M., and Pollack, G. H, Long-range forces extending from polymer-gel surfaces, *Physical Review E* 68, 031408. Copyright 2003 by the American Physical Society.)

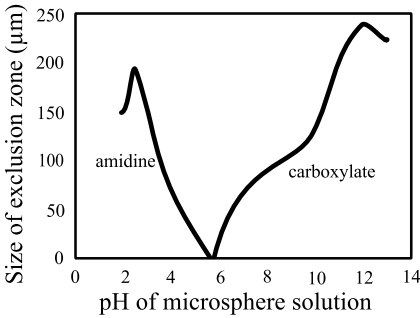


Figure 3.19. Influence of pH on the establishment and size of the water exclusion zone (Zheng and Pollack 2003).

zone was about 100 μm deep. Atomic force microscopy confirmed that the interface between water and gel was clear and sharp. The charge on the spheres was relevant (figure 3.19), but the polarity was not—both positively and negatively charged spheres were equally repelled. Addition of salt and other chemicals had little effect, although the size of the spheres was important—larger spheres can carry a higher charge. This test and others led to the conclusion that solutes are excluded from the vicinity of many hydrophilic gel and gel-like surfaces to extremely large distances on a molecular scale. The structure of the water in the exclusion zone seems to be icelike in that it's ordered—but it isn't ice. Evidence points to its being a fourth phase of water. The structure shows signs of being a solid, as it is birefringent and stable, rather like a liquid crystal. A physical consequence is that it's quite possible (likely, even) that this water is found (used, even) in biological materials, especially load-bearing gels that need to support forces but to do so as cheaply as possible.

3.3.1 GELS

Cellulose is by no means the only polysaccharide of plant cell walls: a host of other polysaccharides, notably the pectins (figures 3.20 and 3.21), form lubricants, gels, and glues at various times in a plant's growth and provide skeletal and lubrication functions, just as polysaccharides are the basis for load-bearing cartilage and lubrication in animals. The functions of such gels seem to be related to the stabilization of water around the plant and the ultrafiltration of ions. For instance, agarose and

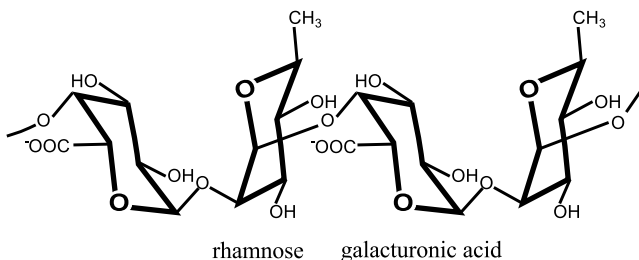


Figure 3.20. Cell wall pectin—the molecules.

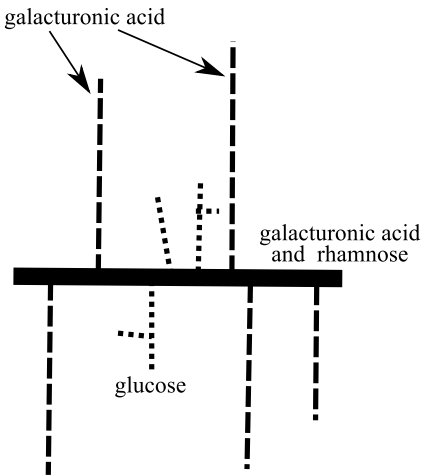
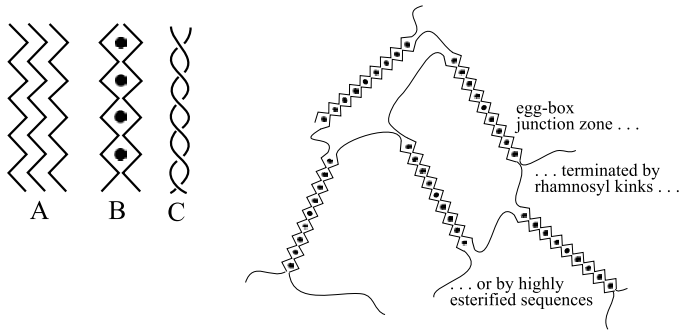


Figure 3.21. Cell wall pectin—the hierarchy.

carrageenan occur in different species of marine red algae as thick extracellular gels that maintain the plant's osmotic environment, provide physical protection, and permit and control the transport of metabolites between the plant and its environs. Some plant gel polysaccharides are extensively used in the food, paint, clothing, and other industries. These are the carrageenans and alginates of commerce—gels extracted from dried seaweed. They have been very well investigated from the biochemical aspect and somewhat researched mechanically.

The chains of gel-forming polysaccharides form extended structures that intertwine or nestle within each other and can be very stable in water, so they must have the characteristics of cross-links of greater or lesser strength. However, in view of Pollack's recent discoveries of the exclusion zone of water and its associated stability, it seems likely that the presence and importance of cross-links in gels should be reappraised and probably downgraded. Current opinion is that the hydration of a gel is controlled largely by its polyelectrolyte nature: the sugar units carry negative charges that bind ions such as sodium or calcium. In agarose, alginates, and pectins two chains nestle, and divalent ions such as Ca^{2+} fill the gaps (figure 3.22) (Mitchell and Blanshard 1974; Rees 1977). These segments provide stability, since the stiffness can decrease by a factor of 4 as $[\text{Ca}^{2+}]$ is reduced from 0.1 to 0.01 M. Current opinion further states that when only a few ions are present, the charges tend to repel one another and push the chain toward an extended conformation that entrains large amounts of water. This tendency is supposedly reinforced by the kinetic freedom of the chains and the associated ions, both of which tend entropically to mix with the solvent as much as possible. However, if the "solvent" is excluding putative solutes with the creation of exclusion zones, this ionic/entropic solvation model will need revision. The possibility then arises that the gel is actually a prestressed structure rather like a plant cell, and the high bulk modulus of water resists the tensile or entropic deformation of the polysaccharide molecules. With alginates the stiffness decreases by an order of magnitude from pH 3 to pH 6; this evidence has been taken to indicate



Calcium pectinate gel

Figure 3.22. Stabilization of gels (Rees 1977).

closer packing of the polysaccharide chains, but increased density of the “connected” phase should lead to higher stiffness. Pollack’s results show that the same pH change reduces the size of the exclusion zone from $200\ \mu\text{m}$ to zero (figure 3.19), which is a large change for what is, in effect, a transition. The fascinating possibility arises that this new model for gels could be the beginning of an evolutionary path for the plant cell: it originates as an unconfined but prestressed gel around which a polysaccharide membrane forms, deriving molecular orientation from its position in an interface between gel and surrounding unbound water.

At the macro level some interesting differences between gels appear to be based on the polysaccharide chemistry. A 2% elastic gel can be strained to 0.2–0.5, so it is not highly extensible (figure 3.23a) (Aizawa et al. 1973). Agarose (figure 3.23b) and agar both show reasonable strength but are brittle. That is to say, a crack once started travels through the material quickly and easily. The question has to be asked, At the molecular level (which is where fracture occurs) what exactly breaks? Is it polymer molecules, or do they rearrange themselves as the exclusion zones are ruptured? Can this question be approached thermodynamically? Dessert jello is also brittle, and one needs only a spoon to break it. κ -Carrageenan (figure 3.23b) shows some ductility—internal rearrangement dissipates some of the applied force. NMR (a technique that gives information about the state of bonding of H^+ within a material) studies suggest that in brittle gels the water is more tightly bound (we might now say that implies a larger exclusion zone), whereas in a ductile gel the water is more free to move, and this allows the chains to adapt to changes in shape. It may be that there is less internal prestrain in such gels.

3.3.2 THE HYALURONIC ACID FAMILY

Whereas plant gels have fairly stable cross-linking sites and are elastic, the related gels (figure 3.24) found in many animals apparently have fewer and more labile cross-links, or smaller or less well formed exclusion zones, since the gels flow under

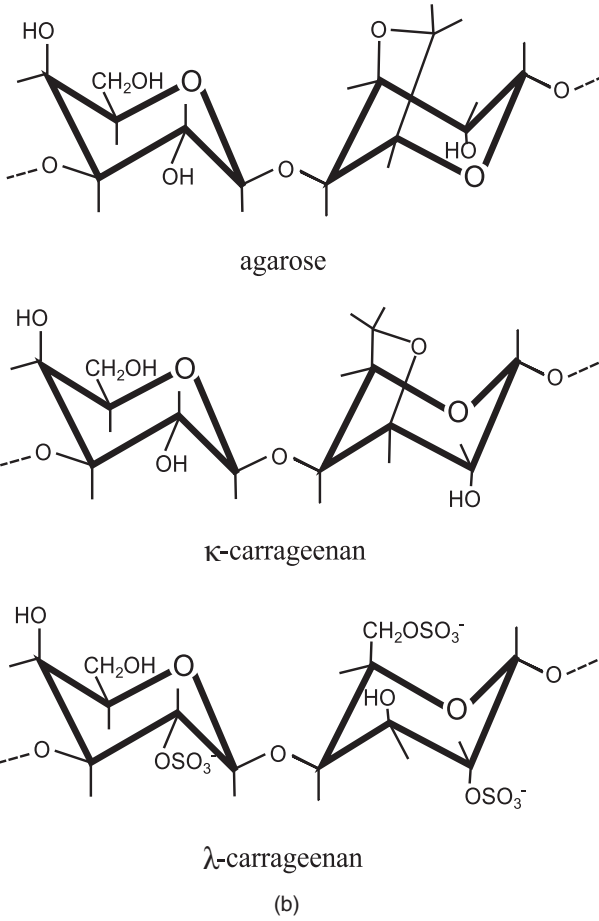
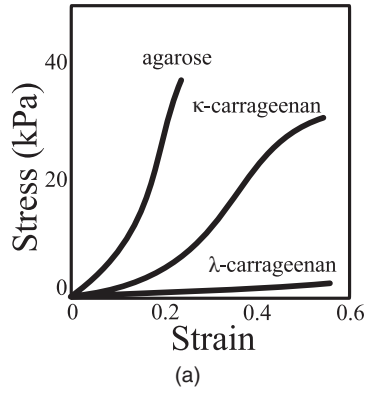
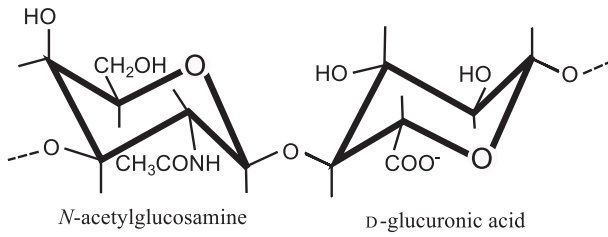
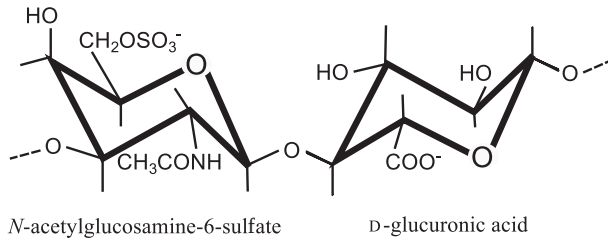


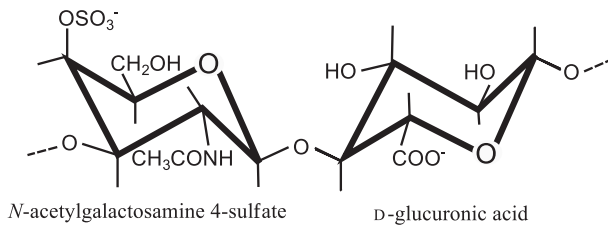
Figure 3.23. (a) Tensile behavior of 2% gels of (b) agarose and carrageenan (Aizawa et al. 1973).



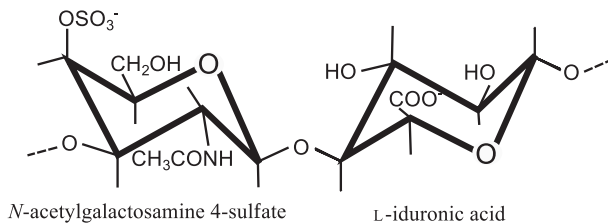
Hyaluronic acid



Chondroitin 6-sulfate



Chondroitin 4-sulfate



Dermatan sulfate

Figure 3.24. The hyaluronic acid family of animal polysaccharides.

stress. Thus the bonds are made and broken and are presumably not so strong as those of the plant gels. The other polysaccharides in figure 3.24 (known collectively as the *polyuronides* or *glycosaminoglycans*) are always associated with proteins. Details of their structure and functions are dealt with in chapter 4. For the present, we consider the properties of the least-charged member of this group, hyaluronic acid (HA).

Hyaluronic acid occurs in a comparatively pure form in synovial fluid, in the vitreous humor of the eye, and in Wharton's jelly (contained in the umbilical cord). It is also found in the vocal folds, dermis, subcutaneous tissue, and cartilage. In all these tissues it influences viscosity, osmosis, shock absorption, wound healing, and space filling. Its molecular weight is probably in excess of 10^6 and may be as high as 10^7 , depending on the source. Hyaluronic acid holds vast amounts of water in its structure and, like any gel, should be considered not as having a direct purpose such as load bearing or lubrication but as organizing the water so that the water itself is then better capable of such functions. To visualize this premise, consider that a single molecule of HA, molecular weight 10^6 , in solution would occupy a sphere $1\ \mu\text{m}$ in diameter. The molecule itself would have a length of $2.4\ \mu\text{m}$. Thus 1 g of HA would occupy 5 L. Table 3.2 shows the degree of interpenetration of HA molecules at various concentrations. Within the zones of overlap the interactions are not so permanent as they are in agar gels, as demonstrated by the increased plasticity of HA. It seems likely that the chain interactions in the cross-linking zones continually change to and from random extended conformation. Such interconversion may well occur in other animal polysaccharides, and the balance between the forces of attraction and repulsion leads to a number of polymers with slightly differing properties for use in the production of different materials.

Hyaluronic acid has a number of interesting rheological features. For example, its solutions are predominantly viscous at low shear rates but predominantly elastic at high shear rates, as evidenced by the crossing of the two lines in figure 3.25 (Kawata et al. 2000). A more objective way of saying this is that the major relaxation time of HA solution is around 4.5 s at pH 6.8, in the absence of salt, at room temperature; that is, HA is viscous when the shear rate is less than $1/4.5\ \text{s}^{-1}$ and elastic above this value (Rwei et al. 2008). The synovial fluid of the knee, which is mostly HA, occupies narrow channels between the soft tissues of the joint, and it is also sandwiched between the two cartilage surfaces. When the joint is flexed slowly, corresponding to the movement with the lower frequency, the fluid moves like viscous liquid in the channels, and behaves as a lubricant. In contrast, under movement with higher

TABLE 3.2
The space-filling ability of hyaluronic acid

% Hyaluronic acid	% Overlap of molecules
0.02	0
0.1	80
0.5	96

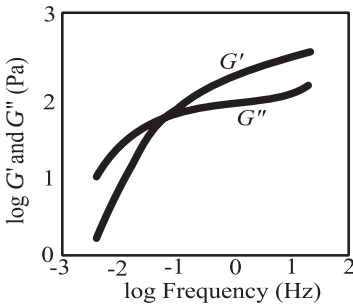


Figure 3.25. Loss and storage moduli of hyaluronic acid as a function of frequency of straining.

frequency, say running, the synovial fluid stays in the channels and behaves as an elastic solid that absorbs the shock of mechanical stress between the surfaces of the articular cartilage and stores the energy mechanically. In pathological joints, the synovial fluid does not have such elastic properties, the crossover of G'' and G' is not observed, and the synovial fluid behaves as a predominantly viscous material over the entire frequency range. The HA is therefore able to flow, and the synovial fluid neither stays in the channels nor stores the energy mechanically against quick movement. Another viewpoint is that the viscosity of HA drops with increasing shear rate up to about 20 s^{-1} , after which viscosity remains constant (Rwei et al. 2008). This drop in viscosity with shear rate (also known as *shear-thinning*) has been postulated to be responsible for the wide range of frequencies produced by the larynx, because the viscosity or stiffness of the vocal folds changes with frequency (Ward, Thibeault, and Gray 2002).

3.3.3 LUBRICATION

Hyaluronic acid sticks to surfaces, altering and controlling their properties. Current theories of biological lubrication require large molecules to be tethered to a surface, as is supposed to happen in synovial joints such as the knee and the hip. As the joint is loaded, a watery solution is forced out of the cartilage. The hyaluronic acid, sitting like the hairs of a brush on the cartilage surface, binds large amounts of water as a gel and provides the separation between the two surfaces, with very low shear strength. The very low sliding friction at natural synovial joints has not yet been attained in any artificial watery joints.

A similar effect has been found with the thin film, less than a nanometer thick, of a high-molecular-weight (2.3 MDa) anionic polysaccharide from the alga *Porphyridium* sp., adsorbed from aqueous solution (Gourdon et al. 2008). The friction with this experimental system was low, and there was no wear even at a pressure of 110 bar and a 1000-fold range of sliding velocities. The lubricant molecules were not squeezed out from between the sliding surfaces, which ensured very low energy dissipation during shearing, but the high mobility of the lubricant, when ordered on one surface, allowed it to move freely. Atomic force microscopy in solution showed that the biopolymer was fixed to the rubbing surface but was mobile and easily dragged

on shearing. The adsorption of this polysaccharide, even to negatively charged surfaces, its stable low friction, its robustness, and the weak dependence of the friction force on its sliding velocity make it an excellent candidate for use in water-based lubricants and as a potential additive to synovial fluid in joints and other biolubricants. That such a thin film can have such a dramatic effect suggests that the water is organized by the polysaccharide—a basis for lubrication very different from the classical thick “polymer brush” layers.

Questions remaining to be answered are: How much of the lubrication is due to controlled shearing of liquid crystalline structures of the water? And how is the shear strength of the water affected by the molecules stabilizing it? Observations on the nature of water at surfaces and the influence on it of biological molecules are of great importance as we enter a new era of understanding of this ubiquitous material.

3.4 Mucus

Mucus has some properties similar to those of hyaluronic acid. However, the example used—pedal mucus from gastropods—is rather more complex than a “simple” polysaccharide gel, since it includes a certain amount of protein as well, though not in fibrous form. For instance, hydrated pedal mucus of the slug *Ariolimax columbianus* is a viscoelastic solid at small deformations (strains of less than 0.1), which implies that the mucus acts as a network. Thus at low rates of deformation (in sinusoidal oscillation, about 0.1 Hz) viscous effects predominate, and there is some flow. At higher rates of deformation (10 Hz) the gel behaves like a rubbery solid. But if the gel is strained to 5 or 6, this network breaks down quite abruptly (yields), and the mucus becomes fluid. The yield strength depends on, and is exponentially proportional to, strain rate, although the yield strain is independent of strain rate, which again suggests a viscoelastic network. Once the shear stops, the mucus heals quickly, the network re-forms, and the mucus becomes solid again. Whether this process is a function of molecular cross-linking or the regeneration of supporting exclusion zones by the water is a question yet to be asked.

The crawling slug employs this solid–liquid cycle as contractile waves move across the sole of its foot (Denny 1984). When part of the foot moves forward, the mucus becomes fluid and presents no resistance. Between the moving waves, parts of the foot are stationary with respect to the ground, and beneath these parts the mucus is solid. Denny calculated a reasonable crawling speed for the slug based on this model, which suggests that the rheological properties of the pedal mucus control the animal’s locomotion, or at the very least are tuned to the other factors involved (e.g., rate of contraction of the pedal muscles). The destruction of electrostatic interactions (e.g., by addition of sodium dodecylsulfate (SDS), a detergent that gives the macromolecules a uniform negative charge) changes the rheological properties markedly (Simkiss and Wilbur 1977). Fresh mucus from *Helix pomatia* shows behavior similar to that of *A. columbianus* (figure 3.26, solid lines). At low rates of shear the mucus is more solid and has a higher viscosity; the viscosity falls as the shear rate increases, finally becoming Newtonian (i.e., viscosity is independent of shear rate), and the

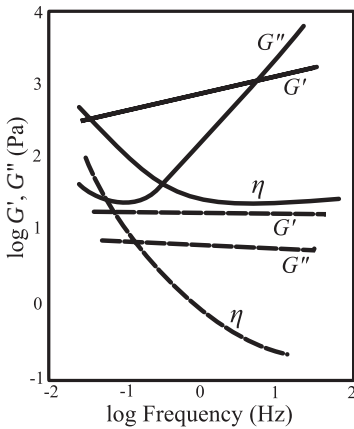


Figure 3.26. Viscoelastic behavior of mucus from *Helix pomatia* native (solid lines) and after disruption by SDS (broken curves). (Simkiss and Wilbur 1977).

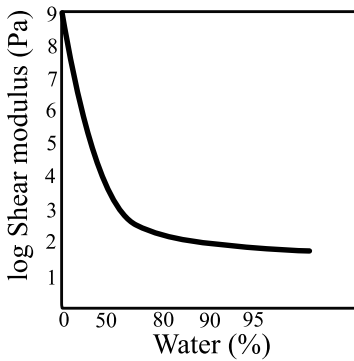


Figure 3.27. Shear stiffness of mucus of *Ariolimax* as a function of water content (Denny 1984).

mucus becomes more fluid. However, the addition of SDS or EDTA, or prolonged storage, changes the behavior drastically (figure 3.26, dashed lines) and reduces both viscosity and moduli. SDS eliminates all electrostatic interactions within and between molecules, which confirms the view that mucus can be considered a transient network formed by the adherence of individual molecules at widely separated points (hence giving a low stiffness). The effect of SDS on the exclusion zone, which presumably contributes to the stability of the mucus, has not been considered. If the mucus is allowed to dry out and the water content drops below 70%, the mucus shows an abrupt increase in shear modulus, confirming the importance of hydrogen bonding (section 3.2). The value rises to about 50 MPa at 10% water content (figure 3.27). The dry material (possibly not totally dehydrated) has a shear modulus of about 1 GPa (the glassy modulus) but still has viscoelastic characteristics. In this condition it makes a very effective glue, although neither theory nor experiment is sufficiently developed to determine whether the effectiveness of adhesion limits the size of littoral organisms exposed to waves (Denny 1984).

Soggy Skeletons and Shock Absorbers

This chapter is mostly about fibers and water. Proteins and polysaccharides are the major classes of structural polymers in biological materials. The number of materials that contain just protein or just polysaccharide is very small. By far the greatest number of biological materials contain both protein and polysaccharide, more or less hydrated and intimately associated. The protein, the polysaccharide, and the water can then be spoken of as phases. In materials science (the main topic of this text) the matrix is the material in which the fibers sit. In biology the term *matrix* is also used to denote any extracellular materials that form a supportive structure for the cells. This meaning of the word is becoming more important in medicine as such materials are manufactured for cells to colonize in the generation of replacement tissues. These matrices are called scaffolds, but in general, their morphology and mechanical properties are subordinated to chemical ones in the process of cell growth and development.

In most animal composites there is a fibrous phase of collagen, and a matrix phase of other protein and polysaccharide. In plants and arthropods the fiber is a polysaccharide, and the matrix is polysaccharide and/or protein. Because water is one of the most abundant materials, with some strange and useful properties, organisms use it as a relatively cheap resource. For organisms living in water, gravity is not a problem, and skeletal structures are mainly required to resist the tension of muscles or the pressure of a hydrostatic skeleton. For organisms living on land, where shape and support are more difficult to maintain, stiff skeletons are more important (chapters 5 and 6).

4.1 Composite Materials

Using a variety of glycosaminoglycans (GAGs) (table 4.1) and three homopolypeptides—poly(L-lysine), poly(L-arginine), and poly(L-ornithine)—as protein models, John Blackwell (Blackwell and Gelman 1975) investigated the nature of interactions between proteins and polysaccharides. Using very dilute (0.0005 M with respect to the monomer residues) solutions, he made mixtures of the polypeptides and polysaccharides and investigated the conformation of the mixtures using circular dichroism (CD) spectroscopy, a standard technique that can detect α -helical conformation. The spectra Blackwell obtained from the mixture were markedly different from those he derived by adding the spectra from the individual components, which suggested that the two components had interacted and that the conformation of one or both

TABLE 4.1
Acid mucopolysaccharide specimens

	<i>Source</i>	<i>Mol wt (kdaltons)</i>	<i>Sulfates per Disaccharide</i>
Chondroitin-6-sulfate (C6-S)	Umbilical chord	40	0.98
Chondroitin-4-sulfate (C4-S)	Sturgeon notochord	12	0.97
Dermatan sulfate (DS)	Pig mucosal tissues	27	1.29
Hyaluronic acid (HA)	Umbilical chord	230	—
Heparan sulfate (HS)	Cow lung	—	0.99
Keratin sulfate (KS)	Cow cornea	16	1.17
Heparin (HEP)	Pig mucosal tissues	11	2.33

Source: Blackwell and Gelman (1975).

of the components had changed. Because the polysaccharide spectrum in the area studied (200–240 nm) is fairly constant and relatively unaffected by changes in pH, salt concentration, or temperature, Blackwell deduced that it was the protein that had changed. He reasoned that if the CD spectrum for the polysaccharide alone was subtracted from the spectrum of the interacting components, the resulting difference spectrum would be for the protein alone. His spectra showed that there is a significant additional proportion of α -helical conformation in the homopolypeptide. With C6-S as the polysaccharide the maximum amount of α -helical conformation is induced in the poly(L-lysine) at a ratio of 1:1, that is, one lysine per disaccharide. Since desulfated C6-S induces no α -helical conformation in poly(L-lysine), it seems that the sulfate groups interact with the lysine side chains. If poly(L-arginine) is used instead of poly(L-lysine), the ratio at maximum interaction is 2:1, which indicates that both the sulfate and the carboxyl groups of C6-S interact with the arginine side chains.

Table 4.2 lists the stoichiometry of several mixtures of polypeptides and GAGs and also shows that the polypeptide is not always in α -helical conformation at the ratio of maximal interaction. The temperatures at which the interactions break down are also given. These temperatures are quite well defined to within a degree or so; this sharpness of melting temperature is typical of noncovalent cooperative interactions. It is also a measure of the strength of the interactions: the stronger the interactions, the greater the degree of thermal agitation (i.e., the higher the temperature) that can be tolerated before the interaction breaks down.

Some of the information from this relatively simple model system can be used to gain insight into the more complex system created by replacing the homopolypeptide with collagen. At the melting temperature of the collagen (observed by CD) the collagen triple helix is disrupted. Does the melting temperature change if the collagen is allowed to interact with the different GAGs? The answer that Blackwell and his colleagues found is yes (Gelman and Blackwell 1974). The change in temperature is not gradual but quantal (figure 4.1), which suggests that the collagen either does or does not form structures with the polysaccharide. Just as in the homopolypeptide model, the interaction with different GAGs is different; table 4.3 shows the number

TABLE 4.2
Stoichiometry of polypeptide-polysaccharide mixtures, conformation,
and melting temperature (T_m) at maximum interaction

Polypeptides		Polysaccharides						
		HA	C4-S	HS	C6-S	KS	DS	HEP
Poly(L-arginine)	Ratio	1:1	2:1	1:1	2:1	1.2:1	1.4:1	3.3:1
	Conformation	A	A	A	A	A	A	A
	T_m	35.0	54.5	65.0	76.0	90+	90+	90+
Poly(L-lysine)	Ratio	1:1	1:1	2:1	1:1	1.2:2	1.4:1	2.3:1
	Conformation	R	A	R	A	R	A	A
	T_m	—	25.0	—	47.5	—	76.5	90+
Poly(L-ornithine)	Ratio							2.3:1
	Conformation							A
	T_{m+}							56.0

Source: Blackwell & Gelman (1975).

Note: A = α -helix; R = random coil.

of disaccharide units per 100 amino acids required to produce a complete change in the melting curve. In each instance all the collagen has been stabilized, and in each case the melting temperature is 46°C to within a degree or so. Thus although the relative amounts of the components needed to form the cooperative interaction vary with the components, the nature of the stabilization is very constant and similar. In fact, the addition of GAGs to tropocollagen in solution affects the formation of collagen fibrils: Blackwell's experiments lend further weight to the idea that such interactions are a controlling factor in the aggregation (or self-assembly) of collagen fibrils in native connective tissues. The complexity of the interactions is further increased by the presence of noncollagenous protein that links polysaccharide chains into larger units.

Blackwell and Gelman did their experiments before it was clear that there are many types of collagen (chapter 3) and that most collagenous tissue is composed of

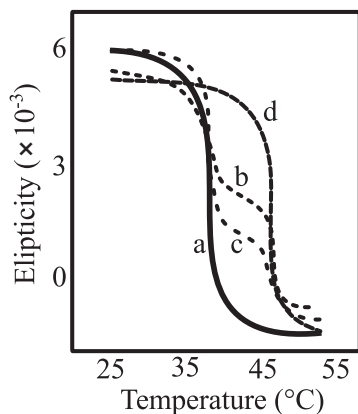


Figure 4.1. Melting curves for the collagen component of mixtures of collagen and chondroitin 6-sulfate: (a) no polysaccharide; (b) 1% polysaccharide residues; (c) 3% polysaccharide residues; (d) 5% polysaccharide residues (percentages are as polysaccharide residues relative to amino acid residues) (Blackwell and Gelman 1975).

TABLE 4.3
Percentage of disaccharide units needed to interact with amino
acids to produce the complete transition of figure 4.1

HA	C4-S	HS	C6-S	KS	DS	HEP
11	14	—	5.5	10	5	—

Source: Blackwell and Gelman (1975).

several collagens. Their experiments confirm that proteins and polysaccharides interact stoichiometrically, which strongly suggests regular structure. It is also now clear that the main polysaccharide derivatives that interact with collagen are themselves combinations of GAG and protein—proteoglycan—in which a core protein has a series of GAG chains attached, and these usually have sulfate and uronic acid side groups. In some instances the proteoglycan can in turn be attached to a central GAG such as hyaluronic acid (figure 4.2) and thus makes up a large molecule capable of filling space as well. The proteoglycan molecules bind together the collagen fibrils into a tissue and transmit force from one fibril to the next (Liao and Vesely 2007). Although the bonds between the proteoglycans and the collagen fibrils are noncovalent and therefore weak, there are many such binding sites, which work additively to transfer force from one fibril to the next. The interfibril matrix is therefore composed of highly hydrated proteoglycans that are dragged along with adjacent fibrils. These proteoglycans experience shearing forces and drag the fibrils closer together and thus interact even further, making the overall tissue stiffer. This shear transfer or shear lag is a well-known approach to describing fibrous composites (Cox 1952) and has successfully been applied to soft collagenous tissues (Jeronimidis and Vincent 1984). The shear lag model is outlined in the next chapter. Although Liao and Vesely appear unaware of the concept, the reduction in lateral spacing of the collagen fibrils that they illustrate (figure 4.3) is a much greater change in distance than the maximum increase in strain (probably about 0.05—Liao and Vesely do not report it), which suggests that the longitudinal Poisson's ratio (see the next section) is relatively large, so

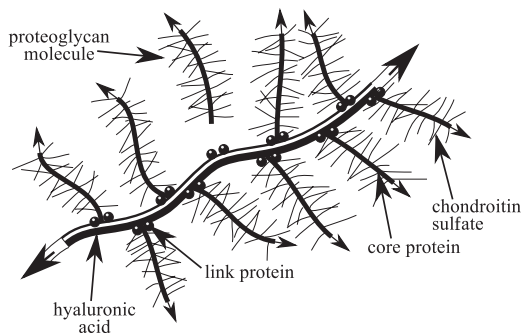


Figure 4.2. Proteoglycan–glycosaminoglycan matrix polymer.

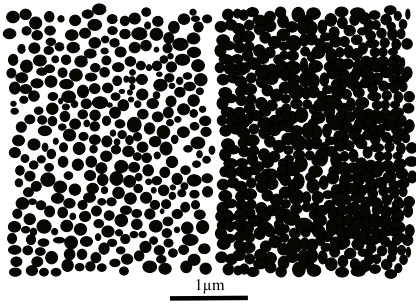


Figure 4.3. Lateral packing of collagen fibers in unstrained (left) and strained (right) collagenous tissue (Liao and Vesely 2007). The position of each collagen fiber is marked with a black disk.

that the fiber they are stretching decreases in volume, like a stretched piece of rope. The collagenous tissue therefore loses water to the surrounding medium whenever it is stretched, and sucks it back in when it relaxes.

4.2 Stress–Strain Behavior

Most soft tissues can be modeled as soft composites of an elastomer (usually mostly water and with a modulus of 1 kPa or less) filled with fibrous and (more rarely, and probably only in sponges) particulate material. An elastomer filled with particulate material will probably be isotropic, whereas one containing fibers is likely to be stiffer along the length of the fibers, depending on their aspect ratio (length:diameter) and the relative amount of fiber present.

Mesoglea and most soft insect cuticles (such as maggot cuticle, but not locust intersegmental membrane, in which the chitin is highly oriented) are examples and show a similar type of stress–strain curve (figure 2.21). Skin, artery, gut wall, bladder, and nearly all other collagenous tissues also show this sort of curve. In these latter tissues it is usually interpreted as follows: the low modulus region represents the stretching of elastin, and the high modulus region is due to the collagen; the collagen contributes relatively little to the low modulus region because it is crimped or folded or just arranged randomly. Apart from the fact that this explanation cannot work for soft insect cuticle, which contains no elastin, there are other ways of obtaining this type of stress–strain curve that hint at some hints of a rather more general model.

A familiar example is knitted or woven fabric (the latter pulled at 45° to the warp and weft). When either material is stretched the increasing resistance to extension is caused by the progressive orientation of the fibers until all of them are oriented in the direction of extension, and the modulus has increased to that of the fibers. This gradual increase in the modulus (the incremental modulus) produces the same sort of stress–strain curve as in figure 2.21. If one considers that the initial orientation of the collagen fibers is statistically distributed, it is apparent that some of the fibers are stressed even at low strains. The progressive increase in preferred orientation of the collagen is then effectively an increased recruitment to a population of load-bearing

fibers aligned sufficiently to support the applied stress and hence the increasing “modulus” of the material (or is it a structure?) (Aspden 1986, 1988).

Two salient facts emerge on examination of this model: first, even at low strains it is possible that collagen fibers can be fully extended and broken. This means that the material is breaking down in a minor way even at low strains; it also means that mechanisms for toughening the material—dissipating the strain energy by limited breaking of the bonds—are available at small strains. Second, at any one time, even at high strains, by no means are all the collagen fibers taking the load: in fact probably only 40% or so of the fibers are bearing load at maximum stress, so that the material can continue to be load bearing even after it has started to break down. Thus any model of soft tissues must take into account not only the amount of collagen present but also the effect of strain on reorienting the collagen.

4.3 Poisson's Ratio and Auxeticism

Some indication of the relative importance of fibers—including their orientation and connectivity—can be gained from studying Poisson's ratio (ν). The principal Poisson's ratio is the ratio between the elongation (the applied strain) and the lateral contraction (in either width or thickness—the induced strain) of the specimen (figure 4.4). The “proper” way to derive the following relationships is to develop the complete mathematical matrix for an elastic homogeneous solid. This matrix includes all the stresses and strains in all three directions, plus the Poisson's ratios, and is the starting point for analytical models of elastic behavior. The following is an abstract of this result, which is a much easier option:

$$\nu_{xy} = \frac{-\epsilon_y}{\epsilon_x}. \quad [\text{Eq. 4.1}]$$

For an anisotropic material (which includes nearly all biological materials, since so many are composites with a preferred orientation) there are six Poisson's ratios. But at present, consider only the second Poisson's ratio in the plane of a sheet of anisotropic material. There must be two, different, Poisson's ratios because

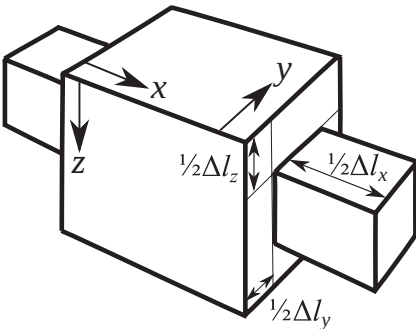


Figure 4.4. Poisson's ratio effects.

$$\frac{\nu_{xy}}{E_x} = \frac{\nu_{yx}}{E_y}. \quad [\text{Eq. 4.2}]$$

If the material is incompressible, then these two ratios add up to unity. This relationship obviously applies to any symmetrical pair of ratios within the material. Because $E = \sigma/\epsilon$, we can substitute strain in Eq. 4.1 to give

$$\epsilon_x = -\nu_{xy} \frac{\sigma_y}{E_y}. \quad [\text{Eq. 4.3}]$$

If the material is being stretched in two directions at once, then

$$\epsilon_x = \frac{\sigma_x}{E_x} - \nu_{xy} \frac{\sigma_y}{E_y}; \epsilon_y = \frac{\sigma_y}{E_y} - \nu_{yx} \frac{\sigma_x}{E_x}. \quad [\text{Eqs. 4.4a, 4.4b}]$$

In a tube under pressure, where the hoop stress is twice the longitudinal stress, we can combine these two equations, but only if the material is isotropic, to allow calculation of Poisson's ratio:

$$\frac{\epsilon_x}{\epsilon_y} = \frac{1 - 2\nu_{yx}}{2 - \nu_{yx}}. \quad [\text{Eq. 4.5}]$$

Otherwise, Poisson's ratio has to be measured independently in the two directions.

Poisson's ratio has often been neglected: it usually appears as a small, constant number in many equations concerning biological materials, and as often as not, its value is assumed to be 0.5, which is the value for any material that retains a constant volume, including rubbers at small extensions. The rationale for this value of Poisson's ratio for soft biological materials is simply that they contain water, and water is (more or less) incompressible. If a rubbery material is isotropic and of constant volume, use of true strain in the calculation of Poisson's ratio gives a steady value of 0.5 or slightly less (because the rubber crystallizes locally at high strains) that is more or less independent of the degree of stretching. Problems occur partly because soft tissues are extended by relatively large amounts (strains of 0.5 and more), partly because they contain fibers that introduce anisotropy unless (unlikely) they are distributed evenly in all three dimensions, and partly because it is not obvious which form of strain is the most appropriate to use for the calculation. For instance, a Poisson's ratio of 1.0 can be obtained from a network or trelliswork such as an orthogonally woven cloth (e.g., a handkerchief) but only in directions at 45° to the warp and weft. The skin on the human belly has similar properties. With a ballpoint pen, draw a chessboard pattern on your belly, then stretch it in various directions and note how similar it is to the handkerchief in the way it deforms. A high Poisson's ratio in all directions is characteristic, if not diagnostic, of an open feltwork rather like a haystack. It is also quite feasible to have an open feltwork embedded in a soggy gel that flows into and out of the mesh whose Poisson's ratio is 1.0 or greater, yet whose volume is constant or nearly so. Such a feltwork has recently been used as a successful mechanical model of octopus skin, consisting of a knitted fabric (from a pair of tights!) embedded in a silica gel.

Thus the assumption that because biological materials contain water and are therefore incompressible it follows that the Poisson's ratio is 0.5 is not tenable. Not only is it possible to have voids in a material filled with "alien" fluids, but it is also possible to have a high Poisson's ratio and constant volume or a vanishingly low Poisson's ratio with a feltwork of fibers embedded in a viscous matrix. And once one starts thinking of different ways of putting a material together, it soon appears conceivable to generate Poisson's ratios of all magnitudes. When a cork is pushed into the neck of a bottle, it doesn't expand sideways, which shows that this cellular material (section 5.5) has a Poisson's ratio close to zero in that direction. If the material expands orthogonally to the direction of extension, it has a negative Poisson's ratio and is termed *auxetic* (Evans 1991). This entertaining phenomenon can most easily be observed in open-cell foams and networks that have been squashed plastically—making some of the walls of the cells reentrant (a three-dimensional version of figure 4.5)—annealed so as to fix the plastic deformation, and then deformed elastically (Evans, Nkansah, and Hutchinson 1994; Martz et al. 1996). But until a proper study is made of the Poisson's ratios of soft tissues under the varying states of initial strain, ν will remain not merely neglected but inscrutable. Worse still, E and G cannot be related with any certainty using Eq. 1.6. Indeed, the true stress and strain that should be used for extensions greater than 0.1 cannot be compressed into a simple formula for calculating E , so that much of biomechanics theory (many equations concerning the mechanics of skin and artery) is wrong, since it assumes that $\nu = 0.5$. It is easy to show that this is not a safe assumption for biological materials.

The principal Poisson's ratio of cow teat skin, stretched as a cylinder by internal pressure (Lees, Vincent, and Hillerton 1991), is totally different from that of rubber (figure 4.6). The teat was lined with a thin rubber sheath (left slack so that it didn't affect the expansion of the teat), mounted on a brass support, and inflated with air. The fiducial marks were indicated by very small spots of ink. The specimens were inflated by small amounts and photographed; the measurements were taken from photographic enlargements. It appears that cow teat skin is in the "open feltwork" category, which is not surprising when sections of the teat are examined: there are many concentric layers permeated with blood vessels and spaces of various sorts. Experiments with isolated strips of teat skin of aspect ratio varying between 1.5 and 10 gave very different results. The Poisson's ratios were negative when the aspect ratio of the test piece was below 2.5; a relatively short specimen gets wider as it is stretched. However, one of the variables was lateral prestrain at the clamps—the skin is very highly folded, and the act of gluing it onto an aluminum tab (which is gripped

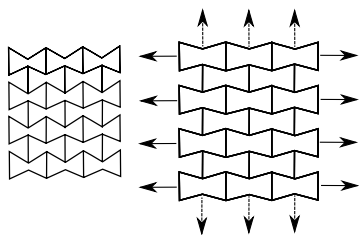


Figure 4.5. Simple auxetic behavior in a plane.

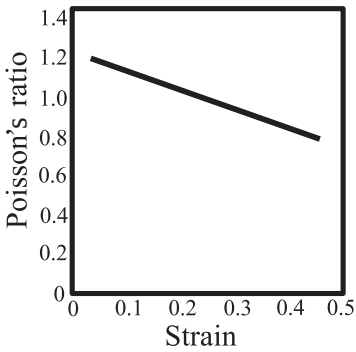


Figure 4.6. Poisson's ratio of cow teat tissue (Lees et al. 1991).

by the clamps of the test machine) flattens out these folds (Vincent 1992). The principal Poisson's ratio of the unrestrained arteries, measured as inflated cylinders, of certain cephalopod molluscs is slightly under 0.5 (Gosline and Shadwick 1982).

Most studies present results of only one of the possible six Poisson's ratios. There is rarely information about changes in the thickness of the specimen. Experimentally, it is very difficult to measure the thickness of pliant materials like skin. It is also possible that the membrane decreases in volume during the experiment, independent of a measured thickness, and that holes in the thickness of the material such as capillaries close on themselves. This is why the skin over your knuckles goes white when it is stretched by clenching the fist. A rope behaves in rather similar fashion: if it is stretched when wet, much of the water is forced out from between the fibers as the volume of the rope decreases. This happens because the rope is a helical fibrous system, and the rotation in the orientation of the fibers when the rope is stretched causes an increase in the Poisson's ratio. Tendon reacts in a very similar way, with a Poisson's ratio at 0.5 or just less over its entire extension (Vergari et al. 2011). Thus Poisson's ratio is an important parameter, and it has to be handled with extreme care.

Biological materials are hierarchical, and it is quite probable that the components at different levels of the hierarchy will have different Poisson's ratios. For instance, materials that are fibrous at the micrometer level may be globular at the molecular level or have a different conformation when stretched, as when α -keratin changes from α to β . An example is the macroscopic response of isotropic fibrin networks in terms of the response of constituent fibrin molecules (Purohit et al. 2011). As the network is stretched (figure 4.7), the fibers rotate from their initial radially distributed orientation to become aligned parallel to the strain axis. The gaps between the fibers disappear, and the network reacts like a rope. At the same time, the fibrin molecules unravel (figures 4.7 and 4.8), and the volume they occupy increases. The Poisson's ratio of the individual fibers goes very high (figure 4.9) as a result of this molecular response to being stretched and so generates significant amounts of internal volume, which presumably draws in water and other components from the surroundings. The material also becomes very compressible for the same reason. The dotted line in figure 4.9 shows the Poisson's ratio for a material of constant volume. Thus as the fibers in the fibrin gel stretch and align, they become thicker and more absorbent. Two

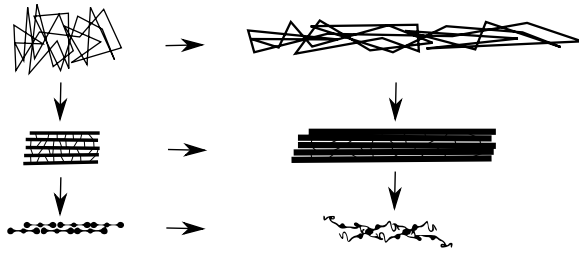


Figure 4.7. Orientation effects at different hierarchical levels in fibrin (Purohit et al. 2011).

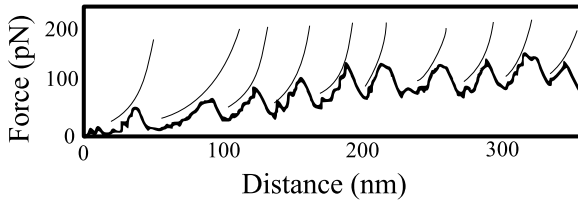


Figure 4.8. “Unraveling” of the globular molecule of fibrin. The force-deflection curves of each segment are sketched in above the data (Purohit et al. 2011).

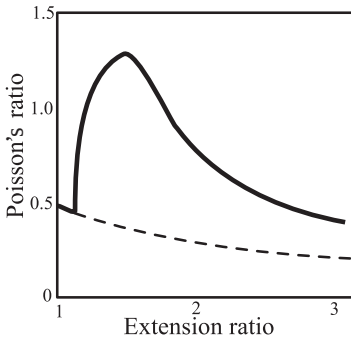


Figure 4.9. Behavior of fibrin in tension (Purohit et al. 2011).

questions remain: What is the strain-related Poisson's ratio of α -keratinous materials when they are stretched and the protein chains become more close-packed? How many other biological materials have these characteristics?

If the fibers in the material are oriented orthogonally to the direction of stretching (figure 4.10), any amount of stretch will not reorient them (in the perfect example) but will cause them to change their position relative to one another. In this instance the Poisson's ratio measured in the plane of the material will be very small (figure 4.11), and the material will deform greatly through its thickness: the locust extensible membrane does this (section 4.5) and has a Poisson's ratio of 0.1–0.02 in the plane of the material, so the diameter of the abdomen of the digging locust does not change very much, but the membrane gets very thin in proportion to the extension. Thus volume is conserved.

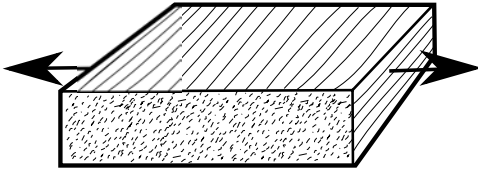


Figure 4.10. Fiber orientation relative to direction of extension of locust intersegmental membrane.

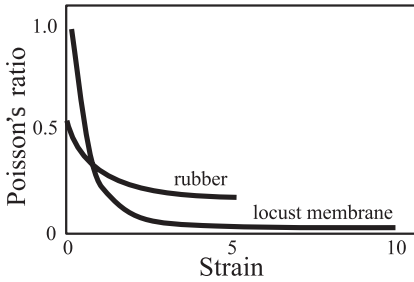


Figure 4.11. Poisson's ratio of locust membrane and rubber compared (calculated using engineering strain).

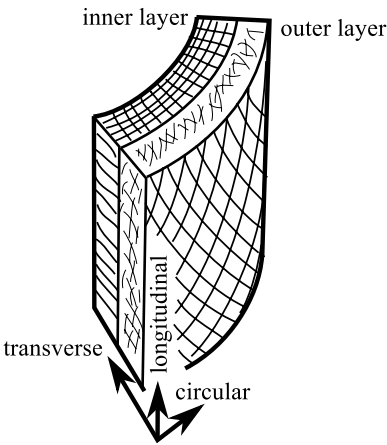


Figure 4.12. Orientation of collagen fibers in the body wall of the sea anemone *Metridium*.

One noteworthy point concerns the analysis of the way *Metridium* expands itself. The explanation given later is that the different stiffnesses interact with the different stresses set up in the cylinder of mesoglea and produce a change in the shape of the animal such that it elongates rather than swells laterally. This is the other side of an argument that could be made based on the Poisson's ratio of mesoglea. This parameter has not been measured for mesoglea, but the nonrandom orientation of its fibers is going to have effects similar to those in locust membrane, where the strain in the direction of the fibers is appreciably less than the strain orthogonal to the fibers when the material is stressed. Thus the outer layer of mesoglea (figure 4.12) tends to deform more through its thickness (gets thinner as it is stretched). Its Poisson's ratio in the length/circumference plane will be about 1.0 whether it is strained longitudinally or circumferentially. But the inner layer will have a very low Poisson's ratio when

stretched longitudinally. How these two ratios will affect each other is unknown, but it is likely that mesoglea is not the only soft tissue that has layers in which the fibers are oriented differently in each.

This discussion of the Poisson's ratio in fibrous biological materials has served only to introduce some of the complexities. For a start, the deformation of biological materials is by no means uniform (Oomens 1985), presumably because the components are not distributed uniformly, either in amount or in orientation. The deformation of hierarchical materials isn't uniform either, especially if they are cellular or have different Poisson's ratios at different levels within the hierarchy (Gaspar et al. 2005). This nonuniformity is seen as increasingly likely with biological materials that have many levels of hierarchy (remember—hairarchy!). Until we find a workable way of dealing, mathematically, with complex heterogeneous hierarchical materials at high strains, we shall continue to discover incongruities that are more apparent than real.

4.4 The Skeleton of the Sea Anemone

An example of a simple connective tissue composed of discontinuous collagen fibers in a mainly polysaccharide matrix is the mesoglea of the sea anemone (Gosline 1971). In one sample of mesoglea taken from eight individuals of *Metridium senile* collected off the coast of California, Gosline found the composition to be salt, 5%; collagen, 6.7%; matrix, 2%; and water, 86%. The matrix material was mainly neutral hexose polysaccharides plus some protein. If allowance is made for the water bound to the collagen, the matrix is a gel containing 2.4% solids, which is well within the normal range of concentration for gels (e.g., a 2% agar gel is very rigid; 1% gelatin gels are stable). The collagen exhibits swelling and birefringence behavior similar to that of rat tail tendon collagen and produces a similar X-ray diffraction pattern. Mesogleal collagen contains rather less proline and hydroxyproline than does rat tail tendon collagen but is otherwise similar. Thus it is assumed that the mechanical properties of the two collagens are also rather similar, with a modulus of around 1 GPa and an elastic strain limit of about 0.04. The mesoglea of *Metridium* strains to 3 or more, so it is clear that the collagen in the matrix is discontinuous and that the main mechanical properties are due to the matrix, modified by the collagen. This modification is influenced by the length and orientation of the collagen and by the strain of the tissue: higher strains tend to orient the collagen so that it bears more of the total load, and the mesoglea stiffens in that direction. However, relaxation rate is as important as strain with a viscoelastic composite such as mesoglea. The relaxation modulus at short times is on the order of 0.1 to 1 MPa. But at long times the equilibrium modulus is closer to 1 kPa, which makes it possible for the animal to stand up to short-term forces such as surge yet inflate itself with the low pressures (1 Pa or less) generated by cilia of the siphonoglyph. When the load is removed from the mesoglea, it recovers, in time, elastically. This recovery suggests that the system is rubbery, which in turn implies the presence of long-chain molecules of high molecular weight. The conclusion that the essential mechanism is rubbery and that the material is working

in the rubbery plateau of the viscoelastic spectrum is supported by the existence of a transition in modulus at about -15°C , at which temperature the storage modulus starts to increase, reaching about 100 MPa at -22°C . This transition is probably due in part to the action of ice crystals that stiffen the matrix.

If the normal state of the matrix is rubbery, then the matrix molecules must have great freedom of movement to result in such a low stiffness. This freedom seems to be due partly to a very dilute matrix, which allows separation of its molecules, and partly due to salts that mask the charged sites on the sugar residues and thus prevent electrostatic interactions that would hinder rotation about the —O— bonds of the polysaccharide backbone. If the mesoglea is washed in distilled water, thus removing the salts and, incidentally, a significant amount of mesoglea (Purslow 1980), the stiffness increases 30-fold; acidic polysaccharides would be too highly charged and more likely to interact. They would also be less likely to form random coils and so to give rise to a rubbery material.

The collagen in the mesoglea does not form a random feltwork; in *Metridium* it is distinctly organized (figure 4.12). The inner layer has a higher volume fraction of collagen than the outer layer, so the collagen may be expected to have a greater effect on the mechanical properties of the inner layer. When the mesoglea is extended longitudinally, the orientation of the collagen fibers in the inner layer is hardly affected by the strain and so has very little effect on the modulus. The outer layer of the latticework is more nearly oriented in the direction of straining, and so it increases the modulus. At a strain of 0.4 or so this orientation is more or less complete, so there is little further increase in modulus at higher strains. Because the matrix then shears past the collagen, the shear properties of the collagen matrix interface become more important. Also, since it is unlikely that adjacent collagen fibers are sheared in the same direction, the shear strain in the matrix becomes greater than the overall shear strain of the material (figure 4.13). This shear amplification increases local shear strain and, because it does not affect the shear modulus, increases the stress required to shear the entire piece of material. Thus the overall effect is an increase in the Young's modulus of the material. This phenomenon is associated with fillers in general, whether fibrous or granular, and is at least partly the cause of the increase in stiffness when fillers are added to elastomers.

When the mesoglea is extended circumferentially, the orientation effect of the collagen in the outer layer is exactly the same as before, but the circumferentially oriented collagen of the inner layer causes an increase in stiffness in this direction. In fact, it is found that the circumferential stiffness is about three times the longitudinal

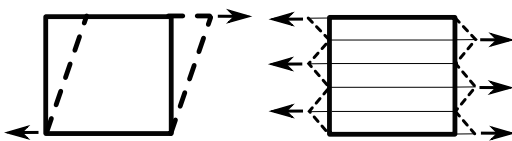


Figure 4.13. Shear amplification due to introduction of stiff elements (here shown as thin horizontal lines).

stiffness. The significance of this difference is that the circumferential or hoop stress of a cylinder under pressure is twice the longitudinal stress. Thus if the stiffness varies in the same ratio, the strains induced by any increase in internal pressure will be the same in the two directions. If the hoop modulus is more than twice the longitudinal modulus, as in the mesoglea of *Metridium*, then as the animal blows itself up, it will extend away from the substrate faster than it increases in girth.

The creep behavior of mesoglea has been investigated, with unexpected results. The retardation spectrum (figure 4.14) shows a discrete distribution of retardation times compared with those of a vulcanized natural rubber filled with carbon black. The narrowness of the distribution also makes it rather risky to derive the spectrum with the Alfrey approximation; nevertheless, it seems highly unusual that mesoglea, modeled as a filled composite, should have such a narrow retardation spectrum. Either the interpretation of the structure of the material is wrong, or a single, very well defined, process is controlling the mechanics. Clearly, this problem needs to be investigated further. One debated explanation is that there are two parallel polymeric systems: one cross-linked that supplies the modulus, and one not cross-linked that supplies the viscosity, both with short retardation times. The steady-state elastic compliance of the viscous system would appear as the instantaneous strain on loading. It has been suggested that at times greater than the retardation times of its components, such a double system would show a very narrow spectrum (Alexander 1962), but this does not explain why such a narrow spectrum is not seen in lightly cross-linked carbon-filled rubbers.

The relationship between the volume fractions of the components of the mesoglea of sea anemones and its mechanical properties is obscure. Within the species *M. senile* there is great variation: the salt-free solids can vary between 9% and 22% of the wet weight. Different areas around the coast of Britain yield animals whose mesoglea has varying mechanical properties but apparently fairly constant composition. Mesoglea of Scottish *M. senile* collected in the Firth of Clyde has more variable mechanical properties than that from English individuals dredged off Plymouth (Purslow 1980). Perhaps these variations are a reflection of the primary type of reproduction—sexual or asexual—that the animals use in the different areas: members of a clone would be less variable in their characteristics. Alternatively, it is possible that there are many subspecies of *M. senile*: How big is a single interbreeding population

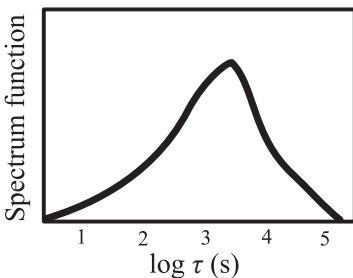


Figure 4.14. Retardation spectrum of mesoglea from *Metridium*, calculated from data of Alexander (1962) using the Alfrey approximation.

of this animal? Problems such as this are no less relevant to the biomechanical approach than to the more common physiological or ecological approach.

Some of the variation between species of sea anemones has been studied (Koehl 1977). *M. senile* lives in relatively sheltered waters; *Anthopleura xanthogrammica* lives in more exposed places. Thus whereas *M. senile* may experience a maximum water velocity of less than 0.2 m s^{-1} , *A. xanthogrammica* may experience two or three times as much. Despite these differences, both species are subject to similar drag forces of about 1 N, since *A. xanthogrammica* is much more squat and streamlined. Mechanical tests on the mesoglea of both species show that *A. xanthogrammica* has a far quicker elastic response after deformation: a force of about 30 kPa extends mesoglea from both species to a strain of 0.6–0.65, so the instantaneous modulus is similar. But *M. senile* mesoglea creeps at a greater rate, and when the load is removed, *M. senile* does not recover so rapidly; it still has a residual strain of 0.1 by the time that *A. xanthogrammica* has recovered completely. If the applied force (in this experiment applied for only a few seconds) is considered analogous to a wave, then *A. xanthogrammica* is not much affected by wave action, whereas *M. senile* is extended farther by each successive wave. If a force of 3 kPa is allowed to act on mesoglea of the two species for much longer times—on the order of a day—then *M. senile* will extend to a strain of 1.5, *A. xanthogrammica* to a strain of only 0.4 or so. In dynamic tests at frequencies between $10^{1.5}$ and 10 Hz, the storage modulus of the two mesoglaes is the same, but *A. xanthogrammica* has a much lower $\tan \delta$, especially at the higher frequencies. This value reflects the quicker recovery response of this mesoglea, since it implies that more of the imposed deformation energy is stored elastically.

To summarize, it seems that the major difference between the two mesoglaes is that *M. senile* has a greater viscous component, which may be due to control of the permanence of the interactions between the components of the matrix and the fibers. If the interactions in *M. senile* mesoglea were relatively less stable, then the differences could be accounted for. Because the instantaneous modulus of the mesoglaes of both species is the same, then the short-time interactions and the units contributing to them must be similar in both. The extreme difficulty of measuring composition, at least of *M. senile*, leads one to question strongly the alternative hypothesis that there is a greater concentration of solids in *A. xanthogrammica*. The published comparisons of composition are based on very few analyses (eight for *M. senile*, three for *A. xanthogrammica*), and the *M. senile* analyses were not of those animals used in the comparative mechanical tests. However, there are differences in the morphology of the mesoglaes. Notably, *A. xanthogrammica* mesoglea has less well orientated collagen that is packed into much larger bundles and may therefore effect the more permanent cross-links demanded by the mechanical data. Another way of creating more permanent cross-links would be to alter the chemistry of the polysaccharides. *M. senile* has mainly neutral polysaccharides, so the possible interactions are minimal. If the polysaccharides of *A. xanthogrammica* mesoglea were more highly charged, then it would be reasonable to expect greater stability of the interactions between them, or more tightly bound water. This study remains to be done.

Sea anemone mesoglea has been considered at some length for a number of reasons. The tissue is relatively simple, so it should be possible (though clearly not yet)

to account for its mechanical properties in terms of its chemistry and morphology. That this has not yet been satisfactorily achieved does not encourage one to have much faith in many of the models proposed for skin and artery, which are much more complex. It is probably also true that the function of mesoglea in the animal is relatively simple: it is generally subject to only two sources of stress—internal pressure that acts slowly, and external water motion that may be continuous (currents) or transient (waves). It is thus not unreasonable to suppose it possible to account for the mechanical properties of the material in terms of the demands of the animal's lifestyle.

4.5 Stretching the Pregnant Locust

Another pliant material whose mechanical properties have successfully been related to the life of the animal possessing it is the highly extensible intersegmental membrane of the female locust (Insecta: Acridiidae). The locust is a migratory desert-living animal and so has to contend with the problems of power/weight ratio (for maximum flight range) and of water supply in general. In practice this means that to reduce its flight payload, the pregnant female carries her eggs in a relatively dehydrated state: they absorb their own weight in water before they develop into nymphs. But to assure for her eggs a reliable supply of water the locust has to lay them deep in the ground (about 8 cm down) usually in the shade of a rock. To reach these depths she uses a highly extensible intersegmental membrane that extends elastically to strains of 15.

The great problem for an animal with an extensible material that is elastic is for its owner not to be caught out by the recoil of the material when the force extending it is removed. For a long time this was thought to be the locust's biggest problem, and some rather messy experiments purported to show that the locust blew its abdomen out like a balloon under internal pressure. But it was subsequently found, simply by making a small hole in the soft intersegmental membrane of a digging locust, that there was no internal pressure, so that the ovipositor assembly at the end of the abdomen must (and indeed does) dig into the ground and pull the abdomen down after it (Belanger and Orchard 1993; Thompson 1986a, 1986b; Vincent and Wood 1972). But what about the recoil problem? It turns out that although the locust does let go of the sides of the hole as it digs, it is safe from catastrophic recoil of its abdomen if the weight of the abdomen (about 1 g) can somehow hold the membrane extended (it takes about 15 g to extend just the intersegmental membrane, discounting the force needed to stretch the internal organs of the locust). The locust does this by stress-softening, a phenomenon fairly commonly observed in biological materials (Vincent 1975). It also was examined extensively in rubber "filled" with particles such as carbon black that interact with the rubber (Harwood, Mullins, and Payne 1965) and associated with the localized increase in strain as the elastomer is stretched around the particles, and bonds form between particle and elastomer. When the strain is reduced, it takes time for the elastomer to adjust these bonds—hence the hysteresis. Given time, the material recovers more or less completely. The phenomenon is therefore an outcome of heterogeneous materials. Figure 4.15 shows the

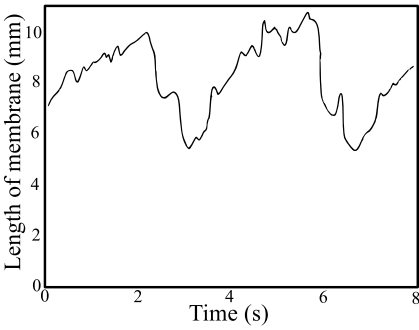


Figure 4.15. Changes in length of locust intersegmental membrane while the insect is digging its hole (Vincent 1975).

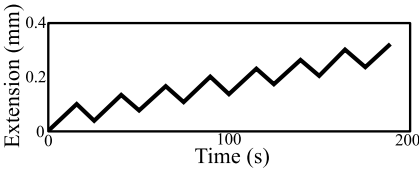


Figure 4.16. Straining program used on a tensile test machine to imitate the strains shown in figure 4.15 (Vincent 1975).

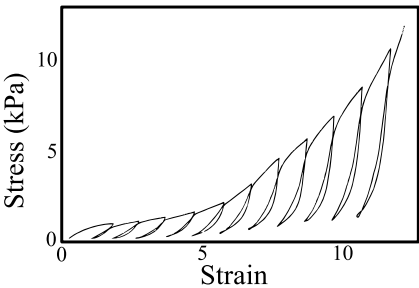


Figure 4.17. Stress–strain curve of locust intersegmental membrane using the strain cycling program of figure 4.14 (Vincent 1975).

strains that an intersegmental membrane undergoes during part of the digging cycle; figure 4.16 shows how these strains were modeled on a test machine; and figure 4.17 shows the response of the intersegmental membrane to this straining program (Vincent 1975). The last cycle shows that with only a very small reduction in length the force required to hold the intersegmental membrane extended can drop by an order of magnitude—from 15 to 1.5 g, about the weight of the abdomen—so there is no need for internal pressure to retain the extension. There will thus be fluctuations in the internal pressure, but in the absence of quantifications of these fluctuations it's not possible to ascribe any function to them (Rose, Seeböhm, and Hustert 2000). This experiment also highlights a general point about the significance of the mechanical properties of biological materials: a host of other experiments on the mechanics of the intersegmental membrane gave some interesting information about the material, but it was not until the test mimicked the way the animal uses the material that the significance of the particular mechanical properties of the intersegmental membrane became apparent. Because biological materials are often designed to accommodate particular working conditions, it is important to know what those conditions are to interpret the mechanics with any degree of insight.

The intersegmental membrane is a type of insect cuticle (Vincent and Wegst 2004). It consists of about 12% protein and 12% chitin, and the rest is mostly water. Despite its higher solids content, the membrane is much more pliant than mesoglea (about 5 kPa, the same as sputum) and has a complex morphology (Vincent 1981) that has probably evolved to contain such a soft material and stop it from oozing away. The chitin plays no part in the elastic recoil properties, since it is arranged at right angles to the direction of extension, but does have the function of a filler; the stretching protein “sees” only the cross section of the fibers rather than their length. The protein must be the elastomer. The chitin controls the Poisson’s ratio and can be seen to buckle along its length as it resists the lateral contraction of the membrane. For the locust, stress-softening is a very important property, since it gives the insect a material that is stiff enough not to extend when the locust does not want it to (e.g., just with the weight of the hanging abdomen) but is soft enough under certain circumstances to be held extended by that same small force. The membrane recovers these characteristics between egg-layings, which occur only once every ten days or so. The abdomen is retracted after oviposition by supercontracting muscles (Herrel et al. 2002).

4.6 Fracture—Chance and Choice

Another aspect of pliant biological tissues that has received little attention for many years concerns how they break or tear. It is obvious that a bone has to be able to resist breaking, as demonstrated by those who have to wear a plaster cast and walk on crutches while their broken leg is healing. But soft tissues are just as liable to fail, and can do so just as catastrophically. For instance, the pilot of an airliner that crashed at Staines a few miles from London’s Heathrow airport in 1973 died during the landing approach (a stressful time for a pilot) because his aorta split longitudinally. This is the preferred splitting direction for the wall of a pressurized cylinder where the hoop stress is twice the longitudinal stress. However, in the healthy aorta it is more difficult to propagate a crack longitudinally than circumferentially owing to the orientation of the collagen fibers. Thus there must have been some pathological difference in the structure of the airline pilot’s aorta to make it act like an isotropic material. What that difference might have been and what caused it is not known. Incidentally, a sausage being cooked is similarly pressurized, so that a cheap sausage with an isotropic plastic skin will split longitudinally, whereas a more expensive sausage, whose skin has been made from the gut of a pig, is unlikely to split for the same reason that a healthy aorta remains intact.

The factors that affect toughness in soft tissues are not very obvious. Table 4.4 lists the toughness values for a number of soft tissues together with their stiffness, and it is obvious that the two properties are not related. Because toughness measures the work exerted on a material to propagate a crack, and work is the product of force and distance, a soft material may require more work to fracture it than a stiffer one, since it can stretch that much farther under the same force (figure 4.18). The fracture toughness of skin, which is difficult to tear, is about $10\text{--}20\text{ kJ m}^{-2}$, an order of magnitude less than that of aluminium foil, which tears very easily. However, it is important

TABLE 4.4
Toughness of some soft biological materials

	Mean tearing energy (kJ m^{-2})	Stiffness (Pa)
Butyl rubber	1.0	106
<i>Metridium senile</i> mesoglea	1.2	105
Pig thoracic aorta	0.98	$\sim 5 \times 10^5$
Rabbit skin	20	10^8
<i>Rhodnius</i> , <i>Triatoma</i> tergal cuticle	1.4	2.5×10^8
<i>Calliphora</i> larval cuticle	1.8	10
<i>Locusta</i> intersegmental membrane	0.2–0.6	10^3

Source: Peter P. Purslow, Personal communication

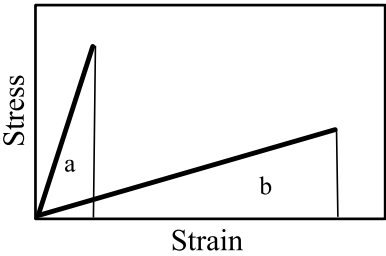


Figure 4.18. Stiffness and ability to store strain energy are not related.

to decide what it means to say that something is more difficult to tear. Is it the ability to take higher loads for a given fracture toughness? Or is it greater extensibility at the same toughness? If one considers only the site of fracture, then the shape of the stress–strain curve doesn’t seem to matter much (Kendall and Fuller 1987), since the elastic—and, more crucially, the strain energy—response of the material is not invoked. Only teeth, scissors, or a knife can produce such effects; thus a blade (as in a microtome) will always register the lowest possible work of fracture (Atkins 2009; Atkins and Vincent 1984). Mai and Atkins (1989) in their riposte to Kendall and Fuller come to the conclusion that biological materials are resistant to rupture because of their low modulus, up to fairly high strains.

Peter Purslow, having been assigned toughening mechanisms in skins as his PhD project—in hindsight, a horrifically difficult problem—survived, flourished, and went on to show that the reorientation of collagen in skin when it is stretched can be greatly affected by the introduction of a short split or notch (Purslow et al. 1984). He used X-ray diffraction to measure the orientation of the collagen. Instead of skin he used a layer from the aorta of the pig—the media. This is simpler than skin in its construction but is still a valid model. With a random arrangement of fibers the material is isotropic, so that initiation and propagation of a crack are equally difficult in all directions.. However, a notch or slit induces a zone of concentrated strain at its tip that decreases very quickly with increasing distance ahead of the tip. Purslow’s results showed that the effect of this strain (detected by the reoriented collagen fibers)

disappeared only 0.5 mm ahead of a 5 mm crack in media that was stretched by about a third of its original length. As the sides of the slit are pulled apart, the local strains are oriented perpendicularly to the long axis of the crack, so that the collagen fibers align perpendicularly to the tip of the slit (figure 4.19). Thus if the slit is to advance, it has to cross many more fibers, each of which has to be broken separately, since they are not firmly glued together. This process requires much more energy and is probably the best way of stopping the progress of an imperfection. We should be pleased that our outer covering is so effectively puncture-proof. This property places skin firmly in the category of a responsive material. Exactly the same response was observed, qualitatively, by Broom (1984a, 1984b), who notched thin sheets of cartilage cut from the femoral condyle at the hip joint of a cow and stretched them. When illuminated with polarized light in transmission, the reorientation of collagen at the tip of the notch is very easily seen, as are the strands of collagen pulled from the torn surface. Thus it appears that the reorientation effect is possible in any soft collagenous composite.

The important characteristics of skins that lead to this type of property are heterogeneity (stiff fibers in a softer matrix) and a degree of mobility of the fibers allowed by the relatively soft matrix (see also section 5.2). Depending on the resistance to crack propagation and degree of initial orientation of the fibers, skins can be stretched to high (10% to 50%) strains that are associated with an elastic modulus that increases with deformation and produces a J-shaped stress-strain curve. This curve seems independently to be associated with high toughness (Mai and Atkins 1989), which is perhaps an underlying reason for such an arrangement of fibers in an outer covering. This association was first pointed out to me by Jim Gordon, who argued

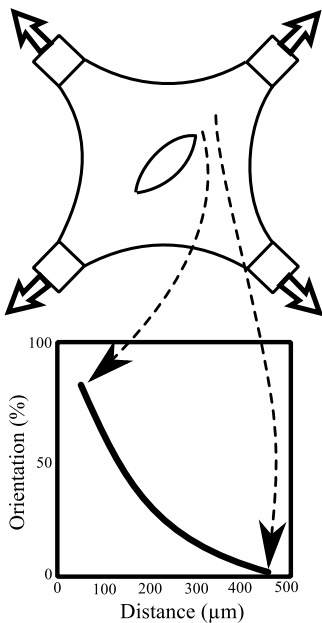


Figure 4.19. Two-dimensional straining of pig aortic tissue showing change in orientation of the collagen due to the strain amplification at the tip of the crack (Purslow et al. 1984).

that materials showing low stiffness at low extensions would not be able to transfer the necessary energy to the tip of a crack to enable it to grow, despite the relatively low work of fracture that has been measured for such materials. Unfortunately, a number of studies, both theoretical and practical, served only to confuse the issue.

To resolve the arguments, Purslow (1989) considered (as the other combatants had done) fracture in mode III—the trouser-tear test (figure 4.20)—which produces force-deflection curves of a fairly consistent type (figure 4.21). In such a test the work to fracture can be calculated simply by measuring the area enclosed by the graph (which represents the work used for fracture) and dividing it by the area cleaved. In this respect the test is very much like the Gurney work-area method (section 1.4) in its usefulness with biological tissues. Purslow maintained that the shape of the stress-strain curve doesn't matter much when the crack is being propagated in the tearing mode, so long as there is sufficient material remote from the fracture zone where strain energy can be stored.

However, when a sheet of material under global tension is being considered, matters are different. Purslow (1991b) used a simple model, based on a power law, that can generate a variety of shapes of the stress-strain curve. The basic formula relates stress to strain as $\sigma = ken$ where k is a constant, and n affects the shape of the curve such that it is straight when $n = 1$, rubberlike when $n < 1$, and skinlike when $n > 1$ (figure 4.22). As his criterion of toughness he used the well-accepted concept of *notch sensitivity*—the tendency for damage, once inflicted, to spread. He then separated stress and strain as driving agents in fracture so that he could decide under what circumstances a nonlinear material with either a higher fracture stress, or a higher fracture strain, might be more “difficult” to break. Starting with a sample whose stress-strain behavior was described by the power law, Purslow established, mathematically, how the elastic strain energy in the sample would vary with notch length and produced some predictions based on straight-line graphs (a

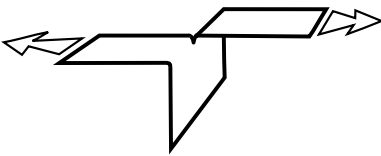


Figure 4.20. Morphology of a trouser-tear test.

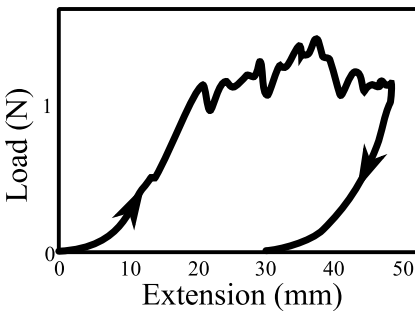


Figure 4.21. Typical result of a trouser-tear test (in this instance, tearing rat skin).

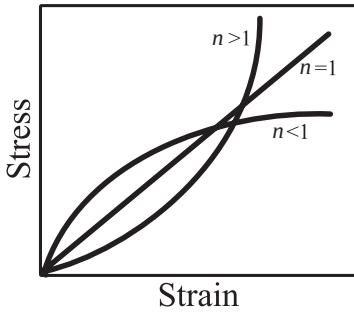


Figure 4.22. Effect of the exponent (n) in a power-law expression for a curve (Purslow 1989).

straightedge is one of science's most potent analytical tools!). In mechanical tests, butyl and silicone rubbers conformed well, and latex rubber less well (possibly because it crystallizes as it is stretched, and the rubber molecules lie against each other with greater regularity).

The outcome of Purslow's work is most easily shown as stress–strain curves (figures 4.23a and b) on which the effect of increasing the length of the initial notch is shown to decrease fracture stress more than fracture strain for a skinlike material, and to decrease fracture strain more than fracture stress for a rubberlike material. These two responses can be shown to work best under two different sets of conditions. The skinlike material is best able to resist fracture under service conditions of high displacement—Purslow uses the example of skin stretched over a knee joint and the high strains experienced in the skin when the knee is bent. The rubberlike curve is probably best suited to applications in which the load is the defining parameter.

Artery and mesoglea have the same toughness, as measured in the trouser-tear test, which illustrates both the conflicting requirements of a tissue and the relevance of various parameters to the actual functioning and design of a biological material. One way of thinking about this dilemma might be to say, “Any biological material

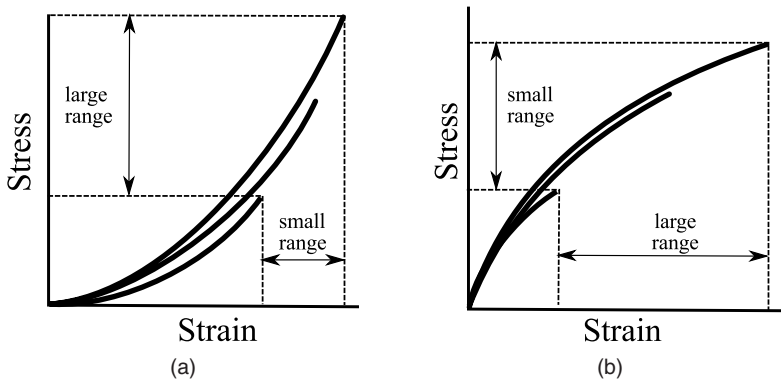


Figure 4.23 (a, b). Illustrating the effect of shape of the stress–strain curve on the relation between these two parameters (Purslow 1989).

is subject to certain forces. Some of these are going to be important and will play a large part in the selection pressures of evolution of the material. Some of these are going to be less important and will not be selected for as rigorously. How much selective advantage does a particular change in the mechanics of a particular tissue confer on the organism? For the one-in-a-thousand chance that an increase of stiffness will allow me to survive longer, is it worth the extra energy involved in modifying the material?"

For instance, it seems highly likely that the toughness of mesoglea comes "for free," probably as a function of reorientation of collagen as a response to damage. The anemone is concerned mainly with the stiffness and time constants of the mesoglea, so it is unlikely that the benefits of the toughness of its mesoglea will be apparent to *Metridium*. The same is true of insect cuticle, although the locust inter-segmental membrane is sometimes found to have been torn and repaired, so it may not be tough (or strong?) enough. But it has to be soft enough for its particular function, and presumably this precludes its being strong. Because this particular material is used only seven or eight times in the life of the locust, it can afford for the design to be close to the working limits and can tolerate a small number of failures, which are not lethal anyway. By contrast, it is possible that artery sacrifices toughness for resilience. The factors that make a tissue tough increase the energy absorption and detract from the elastic performance by emphasizing viscous deformation processes. But part of the function of an artery, especially the aorta, is to store elastically some of the energy of the pulses of pressure in the blood as it is pumped by the heart. The energy is fed back into maintaining the flow of blood between pulses and so tends to damp out the pulses. This damping makes the flow rate more uniform without reducing the velocity of the blood, as might happen if the aorta wall were to absorb the pressure pulses and not feed the energy back into the propulsion of the blood. The aorta is rendered as safe as possible under these restrictions by, as mentioned previously, being tougher in one direction than the other. In fact, it is not possible to measure the toughness of an aorta in the longitudinal direction using a tear test, since the crack tip turns until it is travelling circumferentially. This may happen because the elastin and collagen are both fibrous but are more or less separated laterally and thus thwart stress concentration (section 5.23).

Mammalian skin is a very tough material, for whatever reason. It is logical, however, that the outer covering of an animal should be tougher, and it also happens that skin on some parts of the body (e.g., over the belly) is less tough than the skin elsewhere (e.g., the back). Skin is a complex layered material, made up of four laminae:

1. Epidermis (the surface layer, "keratinized"; see also section 2.3.1).
2. Subepidermal connective tissue (the "grain" layer from which leather is made).
3. Corium (dense collagenous tissue).
4. Hypodermis (looser connective tissue).

The skin, or dermis, usually thought of as the mechanically important part of this structure, is made of layers 2 and 3, which are essentially a more or less random feltwork of collagen and elastin fibers embedded in a protein-polysaccharide (mostly hyaluronic acid and dermatan sulfate) matrix. The mechanical properties of skin vary

over the surface of the animal and are affected by such factors as pH and cross-linking agents, like formaldehyde, that affect the interactions between the components.

Various diseases are due to failure of the cross-links of collagen and elastin. One such is lathyrism, which can be induced by eating the seeds of the grass pea, *Lathyrus sativus*, or its active principle, β -aminopropionitrile, which reduces the formation of cross-links in collagen by inhibiting the enzyme lysyloxidase (Brüel, Ørtoft, and Oxlund 1998). Such diseases have been extensively studied biochemically but hardly touched from the mechanical point of view. They generally reduce the tensile strength of the tissue and increase the extensibility up to 15-fold. Bob Shadwick looked at the skin of the rhino, probably the toughest of all, and its protective function in fighting (Shadwick, Russell, and Lauff 1992). Rhino skin is very thick, with a large amount of large (70–100 μm in diameter) straight collagen fibers arranged in trellis fashion. On the belly the fibers are a little smaller in diameter and slightly crimped. The skin is thus very stiff, with little of the initial toe region on the stress-strain curve (figure 4.24). Clearly, the orientation of the collagen is also important, as shown in a comparison of rhino and cat skins compared with tendon (figure 4.25).

Not all collagenous membranes are tough; some have fracture as their function. Examples are the membrane of the Graafian follicle (Espey 1967; Rondell 1970) and the amnion at birth (Oyen, Calvin, and Landers 2006; Oyen, Cook, and Calvin 2004).

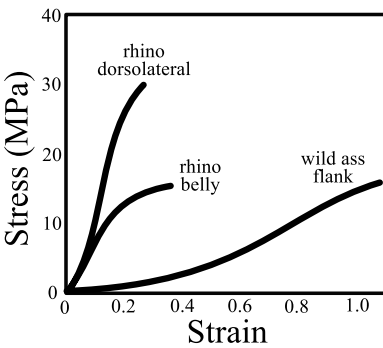


Figure 4.24. Tensile properties of different skins (Shadwick et al. 1992).

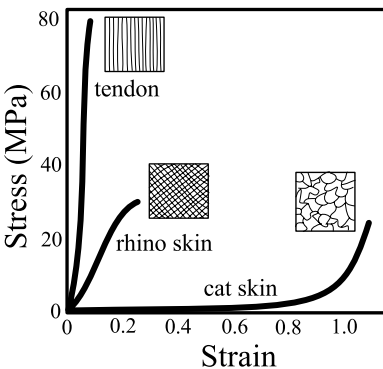


Figure 4.25. More skin, compared with tendon (collagen orientated uniaxially) (Shadwick et al. 1992).

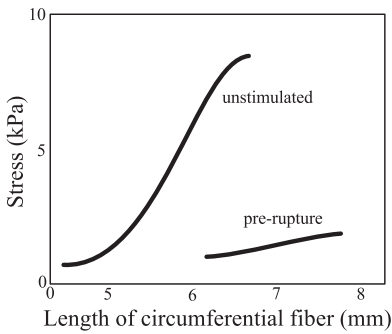


Figure 4.26. Tensile properties of follicle wall, well before and just before ovulation (Rondell 1970).

There are very few published data relating specifically to comparable mechanical properties, although there is plenty of information on the biochemistry and its hormonal control. Just before ovulation the stiffness and strength of the follicle wall drop by an order of magnitude (figure 4.26), and the follicle swells and ruptures. Histological evidence shows that the collagen fibrils in a stimulated prerupture follicle wall are dissociating, which could be a result of breakdown of the matrix or of links between collagen fibers, or of some change similar to lathyrism. The fibrils are fewer and smaller in the wall of a prerupture follicle; in the rat the reduction is on the order of 25% (Morales et al. 1983). The mechanical changes can be induced *in vitro* by luteinizing hormone, cAMP, or progesterone, all at pharmacological concentrations. The suggestion is that these chemicals all stimulate the production of an enzyme from follicular tissue that breaks down the structure of the follicle wall.

The preceding changes are relatively slow. In contrast, similar changes in the intervertebral ligament of the brittle star allow it to rupture little more than one and a half seconds after the animal is disturbed and autotomize an arm as a result (Wilkie 1978). A number of factors can be shown to affect the creep rate and strength of the ligament: K^+ increases both, but its action is blocked by anesthetics, which suggests that nervous mediation is involved. But pH and reduction in Ca^{2+} also affect creep and strength. The hypothesis is that Ca^{2+} is removed from the matrix of the ligament by a glycoprotein in neurosecretory cells adjacent to the ligaments. However, the response *in vitro* is at least two orders of magnitude slower than it is *in vivo*, so presumably some other factor is involved.

4.7 Stiffness—A Biological Variable

One way of describing the action of a muscle is to say that the force of contraction is due to an increase in stiffness. Consider a spring of low stiffness held at constant strain: to hold a stiffer spring at the same strain would require a greater force, or alternatively, the force extending the weaker spring would not extend the stiffer spring so much. Thus if the pliant spring were stiffened but the force holding it extended not increased, the spring would contract (figure 4.27). Echinoderms can work the same trick with collagenous ligaments—the so-called catch-connective tissue—that

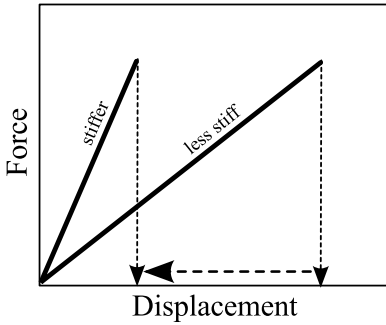


Figure 4.27. A different view of a muscle—as its stiffness changes, it contracts and does work.

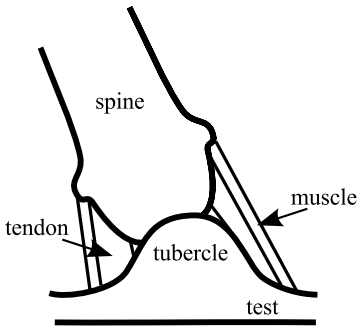


Figure 4.28. Base of an echinoid spine—the muscle is relatively small; the tendon can change its properties.

encircle the base of the spines that cover the body of the animal (figure 4.28). The ligament stiffens when exposed to 10^{-4} M acetylcholine or 10^{-6} M 5-HT, and softens when treated with 10^{-6} M adrenalin or excess K^+ . Muscles are not involved (Takemae and Motokawa 2005).

Holothurians such as *Holothuria scabra* increase the stiffness of their skin by an order of magnitude when they are disturbed (Motakawa 1988). This skin, too, is a collagen-based material. The effect can be mimicked by varying the ionic environment of the skin (Eylers and Greenberg 1989). The modulus of the skin in $CaCl_2$ or distilled water is about 18 MPa, but only 6 MPa in NaCl. The strengths also vary, but strain at break is more or less constant at about 0.4. This reaction seems to be secondary, and the effect of Ca^{2+} variations is to cause cells in the skin to release one or more proteins that have a direct effect on the stiffness (Thurmond and Trotter 1996). One of these proteins has been identified from *Cucumara frondosa* and given the name “tensilin” (Tipper et al. 2002). It binds directly to the collagen and is produced from neurosecretory cells. A similar material, *H-tensilin*, from *Holothuria leucospilota*, another sea cucumber, was found not to affect stiffness very much but to have a marked effect on hysteresis, or energy dissipation, reducing it to almost zero. Thus there must be other components to complete the explanation (Tamori et al. 2006).

A mechanism with some similarities occurs in the spasmoneme of the contractile stalk of protozoans such as *Zoothamnium*, *Vorticella*, and *Carchesium*. The spasmoneme contracts 10 times more quickly and with more power than could an equivalent

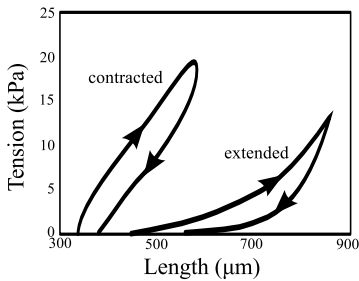


Figure 4.29. Tensile properties of protozoan spasmoneme with and without Ca^{2+} (Moriyama et al. 1999).

muscle and thus pulls the main “head” of the organism down onto the substrate—a defense reaction. Although the contraction was first thought to be due to a rubbery mechanism (Weis-Fogh and Amos 1972), it now appears that the spasmoneme proteins bind Ca^{2+} in a very specific manner, and so the stiffness changes significantly on contraction (figure 4.29) (Moriyama, Okamoto, and Asai 1999). Once the spasmoneme is contracted, no further energy is required to keep it contracted, much as with postural muscle, although the energy saved seems not to have been calculated. ATP is still needed to generate the Ca^{2+} changes, though. Nothing is for free.

4.7.1 PRESTRESS—RIGIDITY FROM WATER

Stiff materials or structures can be generated with or without water. Extras such as calcium salts are required only in the midrange. Because fibers are the main structural motif in biology, it is fairly obvious that stiffness and stability are produced by stretching the fibers. This action obviates many of the problems associated with shear-resistant glues used to hold fibers together and the difficulties with stability when materials are compressed. Nature is very good at making fibers. In plants and animals many stiff structures are made from fiber-wound containers, and on the whole these follow standard engineering design for such structures—the water takes the compression, and the fibers take the tension. The resulting prestressed structure can be very stable and is relatively cheap to make. In animals, the water is usually stabilized within a gel that is surrounded and permeated by a tensile fibrous web of collagen. This form of structure occurs in cartilage (which forms articulating surfaces between bones and forms pliant skeletal elements such as those of the nose, ear, and intervertebral disc). In plants the water is contained in stiff bags and is responsible for the rigidity of unligified tissues (a classic example is the flowering stem of the dandelion). In cartilage the fibrous phase is collagen (plus some elastin), and the matrix is polysaccharide (chondroitins, keratan sulfate, and hyaluronic acid—glycosaminoglycans) stabilized by proteins. This matrix can bind large amounts of water, and just as the turgidity of a plant cell is altered if it is immersed in a hyperosmotic solution that withdraws water from the cell, so does the rigidity of cartilage decrease if the osmotic relationships within it are affected by strong monovalent (or less strong di- or trivalent) cations. Normally, cartilage contains 65%–80% water, 15%–25% collagen, and 1.5%–10% proteoglycan. Thus at the lower end of concentration of solids this

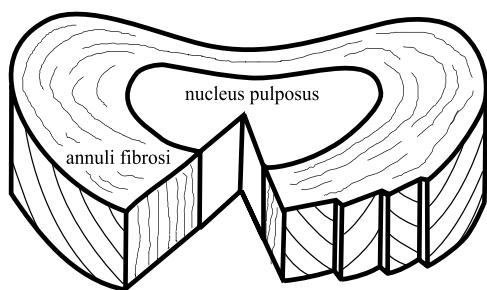


Figure 4.30. Section through an intervertebral disc. The annuli fibrosi develop out of the plane of the section to give a series of short helically wound tubes, nested inside one another.

material is not far removed from *Metridium* mesoglea, but cartilage contains more collagen, and, of course, differs in arrangement and chemistry of its components.

The water-binding of the glycosaminoglycans is a function of the density of fixed charges, just as in gels. In the intervertebral disc (section shown in figure 4.30) the collagen is oriented in a helical fashion, much like that observed in worms and other fiber-wound pressure vessels such as rockets, gun barrels, and cuttlefish. This design allows the layers of the annulus to deform much more readily. Most mechanical testing of cartilage has been on human material from the larger joints, and most has been compressive. Under these conditions the cartilage shows stress-relaxation, owing partly to normal polymeric relaxations and partly to the expulsion of water from the matrix. Stiffness is correlated with the proportion of proteoglycan present. Tensile tests on specimens from femoral condyles show that, as might be expected, the stiffness of the cartilage is also dependent on the content and orientation of the collagen. The impact properties of cartilage are also important, as stresses at the bone–cartilage junction can be as high as 8 MPa just in normal walking. An important function of cartilage is therefore to resist shocks, and so the relative hydration of the matrix and its propensity to lose water under load are important factors. Cartilage probably behaves elastically under these conditions, and the energy in the shocks is absorbed primarily by the muscular system. There is also some evidence that the water content of the matrix is indirectly regulated by mechanically induced electric fields generated during walking and running. In the intervertebral disc system, the end plate differs from the sort found in other joints such as the knee in that it has a tangential layer of coarse collagen bundles arranged in sheets that connect the central area of the cartilage with the nucleus pulposus. The latter provides a stiff core to the intervertebral disc about which the two vertebrae rotate. This stiffness is generated by a gradient of fixed charges built into the glycosaminoglycans as uronic acid and hexosamine (figure 4.31) (Urban and Maroudas 1979). These charged residues cause water to be imbibed into the disc, a process that is balanced by compressive forces on the disc exerted by the weight of the body; restraining muscles, tendons and ligaments; and the restraint due to the collagen annulae of the disc itself. The molal fixed charge densities are about $0.3 \text{ mequiv g}^{-1} \text{ H}_2\text{O}$, which is equivalent to a swelling pressure of about 2 atm. This value is in agreement with the sort of load that has been measured in vivo in the nucleus pulposus of resting subjects. As one ages, the density of fixed charges decreases, and the water content drops, which leads to reductions in

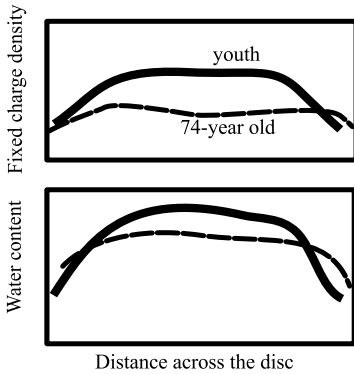


Figure 4.31. Change in the density of fixed charge (upper graph) and water content (lower graph) across a young and an old intervertebral disc (Urban and Maroudas 1979).

prestress in the nucleus pulposus, in load-bearing and flexibility, and in height. Most cartilage has a linear stress–strain curve, but cartilage from the ear has a concave stress–strain curve, which suggests that the collagen fibers in this material undergo greater changes in orientation during straining. Although such a concave shape could also be due to other factors, it illustrates the general point that skeletal materials tend to be Hookean.

4.7.2 DANDELION FLOWERING STEM

Many plants, mostly annuals, rely almost entirely on turgor pressure for their rigidity. This pressure, generated within the cells, can sometimes reach 5 MPa and is reacted against primarily by the fibers in the cell wall but also by other fibers that permeate the structure of which the cell is a part. The flowering stem of the dandelion, *Taraxacum*, is a convenient system to work with (Vincent and Jeronimidis 1992), since very few, if any, of its fibers run along its length, and it has a cellulose gradient from outside to inside. Thus a strip cut longitudinally from the stem will curl by differing amounts when immersed in solutions of sugar varying from 0% to 15%. Also, in a totally wilted stem the epidermis is wrinkled, whereas in the fully turgid stem it is smooth. We can use these observations as the basis for a model if we make the following assumptions:

Proportional to the amount of cellulose in them,

- a. all cells are at the same turgor and have the same osmotic potential;
- b. all cell walls have the same Young's modulus;
- c. all cells are cylindrical along the length of the stem; and
- d. the cells are firmly stuck together.

On the outside of the stem the cells are small ($r = 3 \mu\text{m}$), and the thickness of the cell walls, t , is about $3 \mu\text{m}$; on the inside of the stem they are larger ($r = 30 \mu\text{m}$) with t less than $1 \mu\text{m}$ (figure 4.32). The volume fraction of cell wall material varies more or less linearly from about 0.5 on the outside of the stem to about 0.013 in the inside, with an average of 0.0844. This value corresponds to a mean mass fraction of cellulose

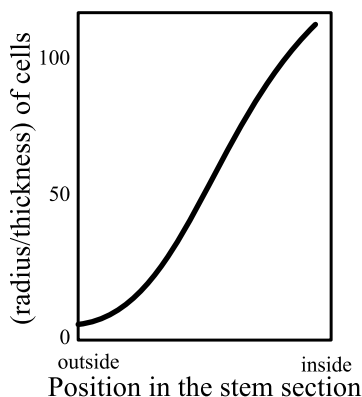


Figure 4.32. Distribution of cell wall material across the flowering stem of a dandelion, measured as the ratio between the radius of the cells and their wall thickness (Vincent and Jeronimidis 1992).

of 8.8% if we assume that the cell walls contain 30% of their water in the cellulose, which itself has a dry density of 1500 kg m^{-3} . This result compares with a gravimetric estimate of dry matter of 8.73%.

The effect of turgor is calculated in much the same way as thermal forces are in the analysis of the mechanics of a bimetallic strip, in which the differential thermal expansion of the component metals causes the strip to bend. In the plant model, the temperature component is represented by the internal osmotic pressure of the cells, and the different metals are analogous to the layers of small, thick-walled cells on the outside of the stem and large, thin-walled ones in the inside. Thus as the pressure increases, the inner cells are liable to stretch more than the outer ones. If a strip is cut from along the stem, the strip will curl more as the pressure increases; an intact stem will not curl because all the “strips” pull against one another and create circular or “hoop” stresses in the outer layers. The concentration of sugar required to cause an excised strip of dandelion flowering stem to straighten has an osmotic potential of about 20 atm, or 2 MPa (figure 4.33). This will be the turgor pressure exerted against the cell walls when the same strip is immersed in distilled water and the cells imbibe water. Calculation based on the bimetallic strip model [Eq. 4.6] gives a radius of curvature of 3.4 mm of a strip immersed in distilled water, compared with an experimental value of 3 mm. Equation 4.6 gives us a quantitative model of the effect of turgor pressure on the mechanical properties of the stem:

$$[\epsilon_x(z)]_{\text{total}} = (BK_x^P/A + \epsilon_x^0)\Delta P/E_{cw}, \quad [\text{Eq. 4.6}]$$

where ϵ_x is the free axial extension due to turgor, z is the position across the stem, A relates the axial force per unit width to the midplane ($z = 0$) strains, B takes into account that in a nonsymmetrical beam the neutral axis of bending is not located at the midplane of the section, P is the turgor pressure (which is what the experiment with the sugar measures), K is the midplane curvature of a strip excised from the stem, and E is the stiffness of the cell wall (Although this is a pretty awful equation for the average biologist, the full derivation [Vincent and Jeronimidis 1992] is really too involved to warrant inclusion here).

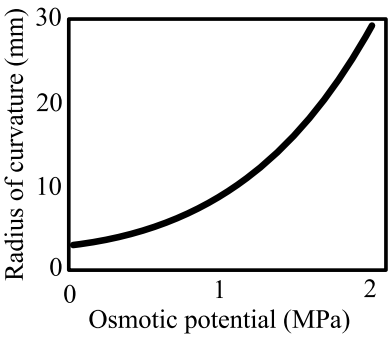


Figure 4.33. Experimental results from incubating strips of tissue cut from the flowering stem of the dandelion in sugar solutions of differing concentrations (Vincent and Jeronimidis 1992).

This model dismisses two of the interpretations of the structure and mechanics of the herbaceous stem that are commonly found in botany textbooks, namely, differences in turgor pressure across the stem are not required, nor are tissues in the center of the stem held in compression in the intact stem. The excised strip curls because the cell walls of the inner cells are much thinner than those of the outer cells. They thus stretch farther under the same turgor pressure, since the stress is greater on the thinner wall. Thus the excised strip curls with the thinner-walled cells on the outside. The resultant strain at any point throughout the thickness of the intact stem is the sum of the strains due to turgor pressure and the bending strains due to the constraints in the stem that hold each excised strip straight (figure 4.34). This strain is independent of position across the stem, which is to be expected, since the stem is straight. We can obtain the net stress in the stem by assuming that for small strains the stress is proportional to the strain, which is shown by a simple tensile test of the intact stem. Thus we apply Hooke's law to each layer and multiply the total strain by the local Young's modulus of the stem. The stiffness of the stem is a product of the stiffness of the cell wall (which is more or less constant) and the relative amount of cell wall material in any one part of the stem (which ranges from 0.5 to 0.013). The stiffness varies across the stem in direct proportion to the volume fraction of cellulose. Thicker cell walls allow a greater contribution to total stem stress, as shown for the dandelion in figure 4.35.

The outcome of this model, in terms of the original formulation of the importance of turgor in the plant stem, is that any compression due to a side load reduces (or is balanced by) the high tensile stress in the cellulose on the compression side. Thus if

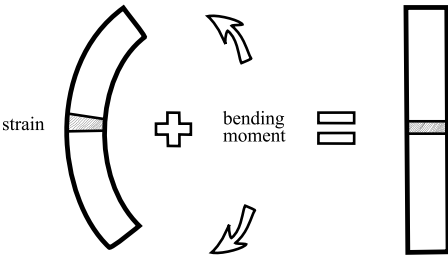


Figure 4.34. Summation of strains in an isolated strip of tissue from the dandelion flowering stem due to the gradient of thickness of the cell walls and the implied bending moment exerted by the constraints of the rest of the stem (Vincent and Jeronimidis 1992).

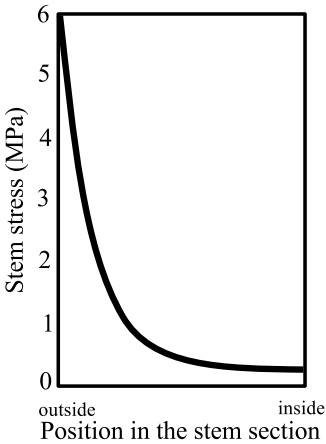


Figure 4.35. The result of the interaction of structure and turgor—very high tensile stress in the skin (Vincent and Jeronimidis 1992).

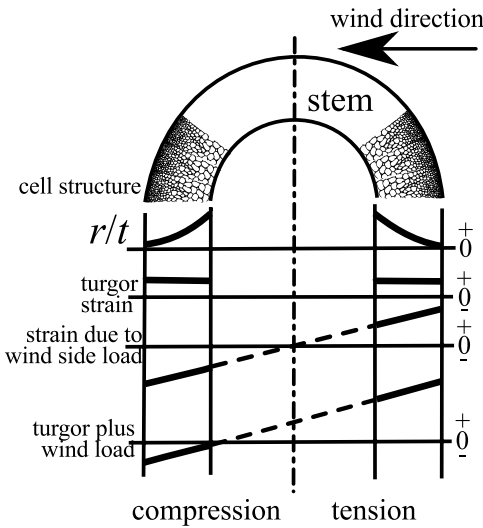


Figure 4.36. Interaction of the internal strain distribution of the flowering stem with an external strain imposed by a wind (Vincent and Jeronimidis 1992).

the plant stem can arrange to concentrate most of its cell wall material at the outer edge of the stem, then the tensile prestress will be at a maximum just where the applied compressive stress will be highest. This situation can be illustrated by summing the strains (figure 4.36), which effectively combines the stresses at the structural and material levels. Admittedly, this mechanism puts much greater tensile loads on the tension side of the stem, but the cell-wall cellulose can withstand loads on the order of 1 GPa before it breaks. An additional advantage of this design is that the small, thick-walled cells that are subject to the highest compressive stress are also much less likely to fail by buckling than the larger, thinner-walled cells found nearer the center of the stem. Thus the gradient in r/t has a twofold effect in resisting buckling of the stem; it acts at both the level of the cell and the level of the whole structure.

Stiff Materials from Polymers

All the materials considered in chapter 4 are more or less pliant, consisting of a relatively small proportion of stiff fibers in a pliant matrix. Under such circumstances the fibers introduce anisotropy (as in sea anemone mesoglea and locust extensible intersegmental membrane) and can resist loads due to internal pressures (cartilage, plant cells) or carry loads along their length (tendons). Fibers are excellent in tension but become unstable and collapse when compressed longitudinally—hence, the impossibility of the Indian Rope Trick. Fibrous structures can be prestrained, for instance, by a turgor mechanism in herbaceous plants or cartilage whereby the compressive loads on the structure can pay off the tensile prestrain. But once the prestrain has been reduced to zero, the material will collapse. A familiar example is a wilting plant. To make a material that can resist compressive loads it is necessary to increase the shear stiffness of the matrix. This will not only stabilize the matrix but increase the transmission of stresses from one fiber to the next and support the fibers against instability. The amount of space available for the change of shape that accompanies buckling will be reduced, so the fiber will be supported in compression. Both these effects will increase resistance to compression. Alternatively, a new phase—glass or crystal, usually opal or calcium carbonate or phosphate—is introduced.

Such stiff materials are useful for a number of reasons. They can provide protection (mollusc and barnacle shells, the skull, sea urchin test), shape and support (diatoms, arthropod exoskeleton), jointed limbs (arthropods, vertebrates), and weapons of various sorts (teeth, tusks). All these materials are subjected to compressive, bending, and shearing loads; however, they have some problems. The fibers are difficult to reorient within a stiff matrix, so they need to be properly oriented and packed right from the start. Additionally, there is the question of effective and safe transfer of loads between the different components, which— independently—can have different stiffnesses and strengths. These mechanical properties have to be matched across the interface that divides them; the advantage is that good matching improves the durability of the composite material. And if biological materials are good at anything, it's in being tough, durable, and able to take physical abuse. Biological materials tend to be “damage tolerant.”

5.1 Crystals and Order

Cells make polymers, polymers make materials, and materials make tissues. Tendon and silk and plant cell walls made their debuts in the previous two chapters, but

nothing was said about how they developed their particular morphology. The cover-all buzzword is “self-assembly.” Once polymers are secreted outside the cell, they find themselves in the company of chemical strangers, all ready for the next bonding session. If biological polymers could not indulge in specific interactions and participate in generating regular shapes, we would not get spots in X-ray diffraction or see birefringence or have anisotropic materials. In other words, we would not have ordered structures with their varied range of mechanical properties.

Many of the biological polymers also exhibit the characteristics of liquid crystals. There is, indeed, good reason to think that without liquid crystals we would not have life, since they are the basis of the organization of molecules and membranes and can be detected in the earliest stages of embryogenesis (Ho 1998; Ho et al. 1996) and may well be the origin of the forces of organization and differentiation that embryologists have sought for so many years. The discovery of liquid crystals can be traced to botanist Friedrich Reinitzer, who showed that esters of cholesterol have two melting points (Reinitzer 1888). Between those two temperatures the liquid was in what is now known as a *mesophase* and was iridescent and birefringent—sure signs of crystallinity and order. Liquid crystalline materials can self-assemble into larger structures, and produce films and fibers without requiring containment or molding, since the molecular chains are stiff (Donald and Windle 1992). In addition, the high degree of orientation of adjacent molecules in a liquid crystalline material promotes insolubility, which gives structural materials an extra durability (and explains why the spider’s web does not dissolve in the morning dew). Although we have only a crude idea of how nature manipulates these molecules, that it makes so many structures using them screams important messages at us. There are three major classes of liquid crystals—nematic (oriented but not layered), smectic (oriented and layered), and cholesteric (also called twisted nematic or chiral nematic, that give the impression of being layered) (figure 5.1). There are also intermediate forms and those that occur under special conditions.

Lyotropic (solvent-based) liquid crystals are excellent for making structural materials in animals and plants (this list is largely due to David Knight) for the following reasons:

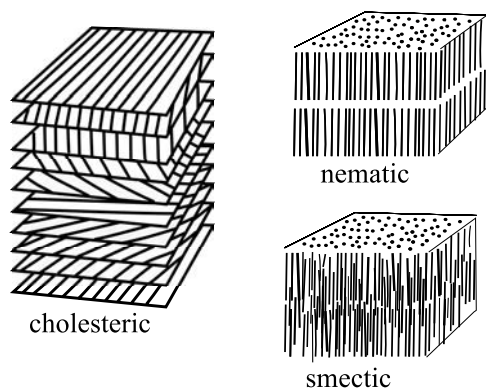


Figure 5.1. Three main morphologies of liquid crystals. There are many intermediate ones that form more complex three-dimensional structures.

1. There is a large range of different assembly states that provide different mechanical and optical properties. Twisted nematic liquid crystals are good for making tough composites (bone, plant cell walls, arthropod cuticle). The more or less parallel molecular alignment in nematic morphology is a good basis for highly stiff materials.
2. Some lyotropic liquid crystals can be modified by “environmental” factors such as concentration, temperature, pH, and salt concentration. These modifications can provide applications involving dimensional changes (e.g., mechanical actuators; striated muscle is a superb liquid crystal actuator), modification of permeability to water and ions to control osmotic effects, semiconductors, switchable optical effects (such as color and reflectance), and biosensors. They reveal many possibilities for the design of responsive materials.
3. Lyotropic liquid crystals readily form hierarchical structures. The trick is to include some impurity to limit the size of the crystallites. Hierarchical assembly is useful for tough materials, as it provides several levels of energy dissipation within a structure.
4. The flexible arrangements in a liquid crystal enable several types of molecules, even from different biochemical classes, to co-(liquid)-crystallize. For instance, many different components of cell membrane, such as phospholipids, cholesterol, and proteins, control its differential permeability by combining into coherent structures. Other chemicals in the resulting liquid crystals can further tune the properties.
5. Some biological liquid crystals self-assemble into ordered structures and then cross-link with hydrogen bonds and/or covalent cross-links (insect cuticle and plant cell walls again).
6. Nematic liquid crystals are highly susceptible to flow elongation. This property probably accounts for much of the structure of collagenous connective tissues and for natural extrusions such as arthropod silks (chapter 2) and dogfish egg cases.

5.2 Composite Materials

The dogfish egg case (otherwise known as the mermaid’s purse, figure 5.2) is a very tough (work of fracture of $6 \times 10^4 \text{ J m}^{-2}$) envelope made almost entirely of layers of remarkably well oriented collagen fibrils (Knight, Feng, and Stewart 1996). The capsule wall, together with its surface coatings and content of jellylike material, is formed as a continuous coextrusion by what is essentially a complex multilayer spinneret (Knight and Feng 1992) known as the nidamental gland (figure 5.3). This gland consists of a series of lamellae organized into six discrete zones of tubular glands, from between which the collagenous material is secreted to form a highly ordered composite sheet of material. The final structure is a series of concentric tubes, each made of a sheet of uniaxially oriented fibers, with a precisely controlled change in fiber orientation from one layer to the next. Relative to the longitudinal axis of the egg case, the fibers are laid parallel, perpendicular, or at $\pm 45^\circ$ and so could be considered a chiral nematic structure. The fibrous part of the collagen accounts for rather less than half the dry weight. Other protein fractions probably represent globular regions of collagen molecules, additional proteins that serve to bond the collagen molecules together, and noncollagenous (essentially phenolic) layers acting as varnishes or glues. Neutral sugars constitute about 0.2% of the dry weight, presumably as polysaccharides.



Figure 5.2. Dogfish egg case or “mermaid’s purse” (from 3 to 10 cm long depending on species).

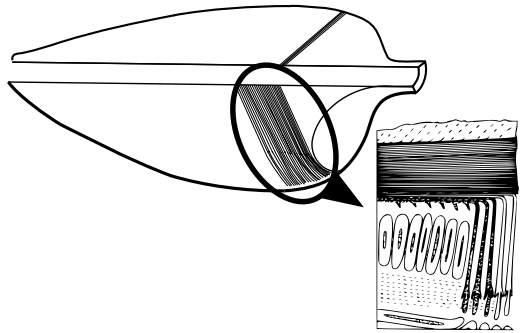


Figure 5.3. The nidamental gland (quartered), which makes the egg case, showing the “spinneret” system that secretes the individual layers (Knight and Feng 1992).

During its secretion, the collagen passes through an extraordinary series of ordered phases, many of which have well-defined liquid crystalline structure. The collagen starts in secretory granules, where it appears isotropic but becomes assembled into a smectic A or lamellar phase. As the granules mature, the collagen passes through a cholesteric phase before adopting a columnar hexagonal arrangement. On secretion the contents of the granules revert rapidly to the smectic A–lamellar and micellar phases. As it passes along the nidamental gland tubules, the collagen is converted into a second distinct micellar phase (Knight et al. 1993). A combination of flow orientation produced in the spinnerets, and the cholesteric arrangement that then appears, defines the orthogonal arrangement of the fibrils of the capsule wall. The take-home message is that the whole structure is crucially dependent on the characteristics of liquid crystalline materials. The same seems to be true for plant cell walls; the cuticles of most, if not all, invertebrate animals; bone—almost any biological material you care to name. In fact, it seems inevitable that all such self-assembled materials should be liquid crystalline, thus showing their dependence on this heritage in the various arrangements of nematic morphologies. The late Charlie Neville produced a definitive catalog of the shapes (1993), although it is by no means obvious how the various arrangements are achieved. The dogfish egg case is probably the best understood example. In plants the orientation of cellulose microfibrils seems to be entirely under the control of microtubules inside the cell addressed to the cell membrane (Smith 2003); in insect cuticle there is a thin layer immediately outside the single layer of epidermal cells that seems to be an assembly zone for the chitin and protein (Wolfgang, D. Fristrom, and J. W. Fristrom 1987). It contains only about 10% solids; the rest is water (Weis-Fogh 1970). Certainly, aqueous suspensions of nanofibers of both cellulose and chitin form liquid crystalline structures.

5.2.1 MATERIAL OR MECHANISM?

At this point in a text on structural biomaterials, the conventional development would be to expound on fibrous composite materials and the models of Cox (1952), Kelly

(1973), and others and to talk of mechanical interactions between the matrix (the in-fill between the fibers) and the fiber. To some extent such discussion is necessary in a consideration of fibrous composites, but it leaves out a swath of biology. In technical composites the matrix is always cured—cross-linked to a degree such that the fibers can transmit high forces to each other via the matrix but not move relative to one another. But even in a “stiff” matrix in a biological material, it is probably rare for the matrix to be truly stiff. Usually, it is plasticized to some degree with water and has to allow for changes in shape as organs and organisms grow and cells elongate and expand. Indeed, in nacre (and possibly some other mollusc shells) it seems that the “matrix” is not a glue but a lubricant! It needs to be hydrated for the material to function properly (section 6.3). Mechanically, the “matrix” is a big problem in plant physiology: how do we separate growth due to stretching from that due to addition of material to the cell wall? Even after one hundred fifty years of experiments with plant cells, stretching them on the rack and increasing their internal pressure, we still can’t separate the effects adequately. All we can say is that the orientation of cellulose produces mechanical anisotropy and hence controls the direction of extension (Suslov and Verbelen 2006). This mechanism is beautifully illustrated in the development of a healing wound, in this case in the root of a pea (Hush, Hawes, and Overall 1990; Hush and Overall 1991), where reorientation of the cellulose changes the shape of the cells and they “grow” or expand toward the wound site (figure 5.4). Reorientation has been observed in real time (Anderson et al. 2010), but the cause is still uncertain. Presumably, it’s still tied in with the liquid crystal model.

Stretching a cell using external force rather than the internal force due to turgor gets a bit simpler. In the literature there is still the feeling that the degree of interconnection of the cellulose fibers—basically the amount by which they can influence each other’s mechanical response—is either close to 1 (there is a comprehensive connection courtesy of a cross-linked matrix with a high shear modulus) or is 0 (fibers act independently in a matrix with low shear modulus). In fact, all gradations are found between these values. The material between the fibers can transfer force directly from one fiber to the next by means of shear (using the concept of transfer length—see later discussion) or by restricting the movement of the fibers by specific micromechanical

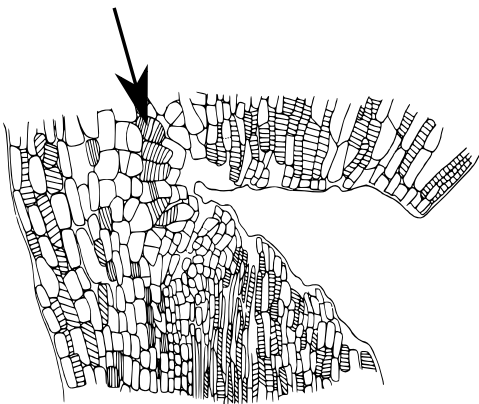


Figure 5.4. Part of a pea root that was cut 24 h previously. Cells at the tip of the cut (arrow) have rotated the orientation of the cellulose in the cell wall and are expanding toward the cut. (Adapted with permission from Hush, J. M., Hawes, C. R., and Overall, R. L., 1990, Interphase microtubule re-orientation predicts a new cell polarity in wounded pea roots, *Journal of Cell Science* 96, 47–61.)

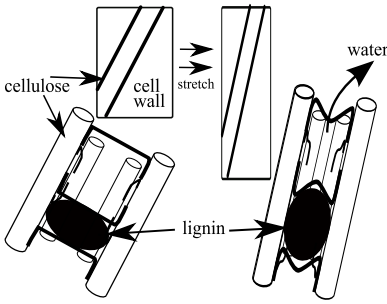


Figure 5.5. Partial stabilization of cell wall by lignin. The components are not constrained from moving relative to one another, so the cell wall becomes a mechanism (Hepworth and Vincent 1998).

interactions. This latter model for interaction (figure 5.5) was developed to account for the stiffness of cell walls with a low degree of lignification (Hepworth and Vincent 1998) and provides a much better description of the mechanical properties (figure 5.6). The driver is that cellulose microfibrils winding around a cell (a common morphology in lignified tissues) get closer to one another as the cell elongates (Poisson's ratio again) and squeeze and shear whatever is between them.

Thus we arrive at the idea that adjusting the amount, and shear stiffness, of the matrix can produce a variety of effects not found in conventional technology. A well-known example is the modulation of stiffness by the addition or removal of water, especially in bilayers. A simple experiment reduced a top-level conference (in which most of the presentations described work using state-of-the-art equipment) to open-mouthed awe (personal experience). It happened like this. Most paper is produced on a machine that orients the fibers parallel to the direction of travel of the sheet—the “machine direction.” The paper is thus slightly anisotropic and when wet will swell slightly more in a direction orthogonal to the fibers. This property is sufficient to cause an appropriately prepared bilayer (figure 5.7) to curl when wet, an

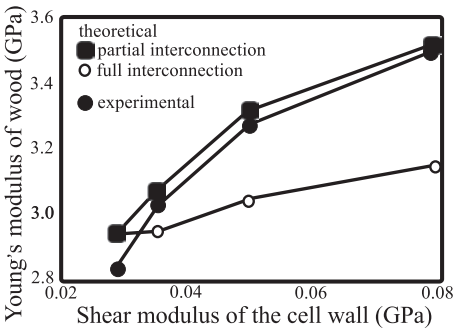


Figure 5.6. A comparison of the model in figure 5.5 with a standard composite of fibers cross-linked into a matrix. Experimental data strongly support the model with partial interconnection (Hepworth and Vincent 1998).

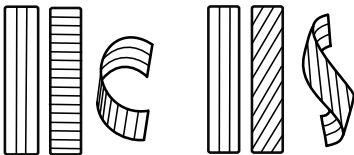


Figure 5.7. Hydration effects on bilayers. If the first two strips in each of the sets of three are stuck together in the orientations shown, the resultant strip when wet will curl like the third figure in each set.



Figure 5.8. The awn of *Erodium* (a geranium) showing a helix induced by change in moisture (Strasburger et al. 1903).

experimental demonstration that requires only a glass of water. This basic phenomenon is found in the awn of *Erodium* (a geranium), which curls and uncurls (Figure 5.8) with diurnally changing moisture (e.g., dew) and literally screws itself into the ground (Strasburger et al. 1903). The same basic mechanism is responsible for seed dispersal in many plants (Witztum and Schulgasser 1995), where it is allied to brittle fracture when the matrix dries out (Vincent 1990).

5.2.2 FIBROUS LEAVES

Separate fibers occur at higher levels within the hierarchy of structure. In grasses, the sclerenchyma fibers are nearly 1000 times stiffer than the parenchyma tissue between them. Thus although only about 5% of the leaf is composed of such fibers, they contribute about 95% to the overall stiffness of the leaf (Vincent 1982). Information about the ability of the grass leaf (and other materials with parallel fibers) to resist damage due to grazing or being trodden under foot can be gleaned by testing the strength of a test piece that has been damaged in a controlled manner, such as by the introduction of a notch or cut. The notch directs the fracture so that the test piece does not break at the clamps; depending on the material, the notch may also cause a stress concentration. In a plot of tensile strength versus the relative length of the notch (expressed as a fraction of the total width of the specimen), a straight line (figure 5.9) indicates a “notch-insensitive” material that can sustain damage without being greatly weakened. In other words, the strength of the material is a simple function of cross-sectional area. Grass leaves and the lamina of the seaweed *Laminaria* fall into this category (Vincent 1982; Vincent and Gravell 1986). If the line falls lower (figure 5.9), it indicates that the material is “notch-sensitive” and can be weakened by the presence of small imperfections. This sensitivity is potentially dangerous for the integrity of the material, since a small crack may severely weaken the material (section 1.4). With up to about 10% by volume of sclerenchyma, grass leaves are almost completely notch-insensitive, most probably because the shear stiffness of the cells between the fibers is relatively low. Thus even if several fibers are broken, stress is not sufficiently transmitted laterally to cause a stress concentration in the remaining fiber(s), and a notch will not weaken the leaf significantly. This is not so with *Stipa*, which has a continuous sclerenchymatous layer over the upper surface of the leaf, which contributes about 30% of the leaf tissue (figure 5.10). A crack can propagate through this layer, and the *Stipa* leaf becomes notch-sensitive (figure 5.10) (Vincent 1990).

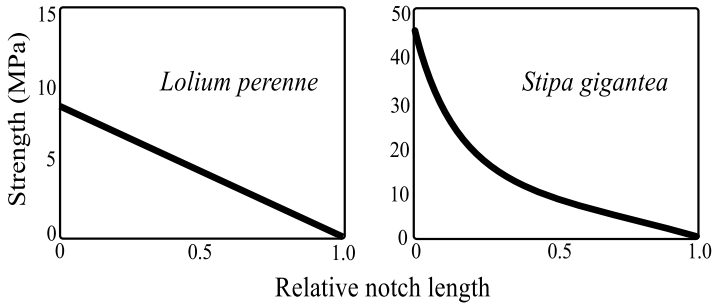


Figure 5.9. Sections of grasses. *Lolium* is very soft; *Stipa* is vicious (Vincent 1982, 1991).

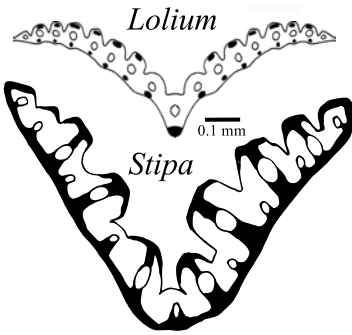


Figure 5.10. Notch sensitivity of *Lolium* and *Stipa*. Large amounts of fiber make *Stipa* strong, but it is more susceptible to damage (Vincent 1982, 1991).

The notch-insensitivity of the softer grasses has a number of important consequences for animals that feed on it, since it means that teeth are of little use other than for gripping the grass. Like meat (and for the same reason), it must be torn by brute strength or cut with a shearing action (Purslow 1991a). Large animals such as cows and sheep hold the grass (respectively with tongue and teeth) and pull. They thus are limited in the number of leaves they can break, as illustrated by considering the effects of “enrichment” of pastures. Longer grasses are harvested at least as easily as shorter ones, so it is worth encouraging grasses to grow tall. But if the tillers grow more densely, the cow or sheep, having finite strength, will find the size of its bite reduced, even though it might be taking in the same number of grass leaves. This reduction in bite size results because the strength of grass and its apparent stiffness (as measured from the total cross-sectional area of the leaf) are directly proportional to the amount of fiber present. Thus increasing tillering will increase intake only up to a point. A number of different studies have shown an inverse correlation between the strength of grass and “palatability” (Theron and Booysen 1966), so that the weaker grass is, the more the animal will eat. It has also been pointed out that when deer

age, their teeth tend to rot. If their back teeth remain, food intake is not impaired. However, if their back teeth decay, they cannot survive, even if their front teeth are in good condition. Clearly, deer do not need their front teeth for gathering grass but do need their back teeth for comminution. Smaller animals such as rabbits have to cut through the individual fibers of the leaf, since they are not strong enough to break the grass in tension. Smaller still, locusts, and caterpillars do not bite all the way across but at some point bite down between the fibers and cut their way out again across the fibers. Mechanical problems with harvesting food have been confused with behavior: Soay Island sheep meat is famed for its flavor—it comes ready salted because the sheep eat seaweed along the shore line. For a long time their aversion to large fronds of *Laminaria* was ascribed to a mythical antifeedant. But the sheep ate the stuff readily when it was chopped up for them; they simply couldn't bite off bits small enough to chew (Vincent and Gravell 1986)!

As grass dries, the stiffness increases, but the work to fracture changes hardly at all (Vincent 1983). At a water content of about 0.2 g per g dry weight of grass, the fracture properties of the cells between the fibers go through a transition, and these cells become very brittle and weak. The grass then tends much more to fracture between the fibers, and to do so in a very brittle fashion. This seems to be the condition for hay-shatter; hay containing 25% or so of water is considered to be suitable for bringing in from the field. Hay with a lower water content is considered to be very susceptible to shatter, with consequent high losses of the crop. Water content has been shown to be the major factor in hay-shatter in nongrass hay species (Shepherd 1961).

5.2.3 LOCUST TENDON

Eventually, it becomes apparent that organisms need stiff, durable, waterproof materials, and it is in these areas that composite theory greatly helps understanding (Ward and Hadley 1998). There are two limiting cases (figures 5.11 and 5.12). In the simplest one the fibers are pulled along their length (isostrain model—the fibers and matrix are stretched the same amount), and the stiffness is a simple sum of the stiffnesses of fiber and matrix multiplied by the amount of each:

$$E_c = E_f V_f + E_m (1 - V_f), \quad [\text{Eq. 5.1}]$$

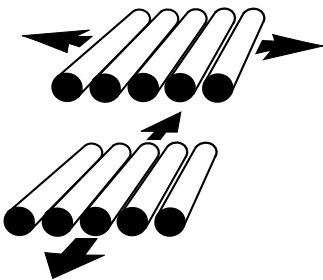


Figure 5.11. Two basic morphologies for composites—pulling along or across the fibers, which are embedded in a matrix.

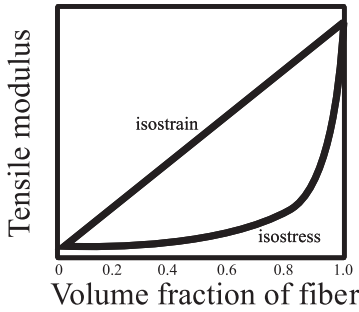


Figure 5.12. Variation of stiffness with the amount of fiber present, dependent also on its orientation.

where E is the stiffness, V is the volume fraction of any component (here there are two components, so the two of them add to unity) and c , f , and m are subscripts indicating composite, fiber, and matrix, respectively. This stiffness is thus a weighted average modulus of the two components and is also known as the *Voigt average modulus*. The other limiting case—in which the material is pulled orthogonal to the fibers (isostress model)—is a bit more complex and is known as the *Reuss average modulus*:

$$\frac{1}{E_c} = \frac{V_f}{E_f} + \frac{(1 - V_f)}{E_m}. \quad [\text{Eq. 5.2}]$$

The derivation of this equation is commonly found, and is in Ward and Hadley's book (1998).

Insect cuticle can be analyzed as a classical fibrous composite (Ker 1977), although the analysis has rarely been attempted (Vincent and Wegst 2004). Ker investigated the apodemes from the hind leg of the locust *Schistocerca gregaria*, which are particularly convenient for a study of this sort. They are designed for tensile straining and are therefore appropriate for tensile testing; the average direction of the chitin microfibrils is parallel to the long axis with very little variation; the apodemes are highly uniform over relatively long distances; and there is considerable information on their structure. It is therefore possible to analyze the mechanical properties of such tendons using the theory established for fibrous composite materials. We then need to determine how far the theory fits with the observed results.

First, we consider the detailed morphology of the material: calculations relating electron micrographs of tendon to the chemical composition show that the microfibrils observed in cuticle are indeed chitin, that the matrix is protein, and that the two phases are separated. The chitin is strongly H-bonded, so much so that it is insoluble in boiling 1 M NaOH. There are several models for the way in which the proteins are bonded to the chitin, but the most reasonable harks back to early work on chitin (Fraenkel and Rudall 1947), in which its OH groups were observed to be arranged with the same periodicity as the amino acids in a β -sheet conformation. This model has been amply confirmed by Judy Willis (Iconomidou, Willis, and Hamodrakas 2005). Thus the matrix is very well bonded to the chitin fibers. Evidence for this structure is provided by the brittle failure of the preferred cuticle when it is pulled

longitudinally—the fracture surface mostly goes straight across the sample with little or no evidence that the fibers are pulled from the matrix. It's educational to compare chitin with conventional fibers in fiber composite materials. The minimum allowed diameter of carbon fibers is 5 μm , since smaller fibers are medically dangerous if breathed in. Chitin nanofibers are typically about 3 nm in diameter, but only half their surface has exposed H-binding sites, so they present 10^6 times more surface area than carbon fibers for interfacial interactions with the matrix per unit volume. Chitin composites are therefore tough (a function of the interfacial bonding area). Furthermore, chitin is a component of our metabolism—edible composites!

The longitudinal Young's modulus of the locust tendon is 11 GPa; the transverse Young's modulus is 0.15 GPa. The volume occupied by the chitin in the whole material (the volume fraction of the chitin) is estimated as 17%. The difference between the two moduli is due to the extreme degree of orientation of the chitin fibrils.

$$E_c = E_f V_f(z) + E_m(1 - V_f). \quad [\text{Eq. 5.3}]$$

Equation 5.1 is modified slightly to account for the fact that the fibers are not completely tightly bonded with the matrix and, being stiffer, are therefore likely to develop shear gradients in the matrix material along their length. It is this shear that transfers the loads from one fiber to the next. The quantity z is a complicated function (otherwise known as a “correction factor”) that includes the stiffness, cross-sectional area, radius, spacing, orientation, and length of the fibers as well as the change in shear in the matrix caused by the presence of the fibers. See Eqs. 5.4 and 5.5. Kelly (1973) gives the full derivation.

$$z = 1 - \left[\frac{\tanh ax}{ax} \right], \quad [\text{Eq. 5.4}]$$

where a is the aspect ratio (length/diameter) of the fibers, and x is given by

$$x = \left[\frac{2G_m}{E_f \ln(R/r)} \right]^{1/2}, \quad [\text{Eq. 5.5}]$$

where G_m is the shear modulus of the matrix, E_f is the Young's modulus of the fibers, R is half the separation between fibers, and r is the radius of the fibers. This expression averages the mechanics of a single fiber embedded in a cylinder of radius R over the entire composite. The two key ratios are G/E , which is typically 0.01 to 0.02, and R/r , which should be not much greater than unity. Using this model, Ker deduced that stiff arthropod cuticle, as exemplified by the locust tendon, conforms to the behavior of a fibrous composite material and yields the data shown in table 5.1.

The estimate of the stiffness of chitin given in table 5.1 is very low (the true modulus of chitin is probably in excess of 180 GPa). The estimate for the stiffness of the protein matrix may be typical, but the stiffness of the matrix can vary over seven orders of magnitude (Vincent and Wegst 2004). If the elongation of a chitin nanofiber is 2% at break (which is half the [doubtful] published value), then its strength is 3 GPa. If the strength of a hydrogen bond between the nanofiber and the matrix protein is 20 pN with an attachment area of 1 nm², then the interfacial shear strength is 10 MPa,

TABLE 5.1
Mechanical data for the components of insect cuticle used by Ker (1977)

Stiffness of chitin microfibrils	70–90 GPa
Stiffness of protein matrix	120 MPa
Length of chitin fibrils	0.36 μm
No. of NAG residues in a chitin fibril	~700
Aspect ratio of chitin fibril	~120

and the critical transfer length is about 0.5 μm (Vincent and Wegst 2004). For a fibril to be reinforcing (i.e., twice the “transfer length”; see later discussion), it must be at least 1 μm long. This estimate of the length of chitin fibril derived from mechanical considerations is interesting, since it is of the same order as that obtained from the cuticle of *Sarcophaga* (larva, puparium, and adult) using gel filtration techniques (Strout, Lipke, and Geoghegan 1976).

The discontinuity of chitin fibers further complicates the factors involved in the mechanics of cuticle. The mathematical models assume that forces are not transmitted to the fibers across their ends but are transmitted by shear along a defined length—the transfer length—of the fibers. Thus the shear stress at the fiber–matrix interface has a maximum at the ends of the fiber and a minimum at the center; conversely, the tensile stress in the fiber is minimum at the ends and maximum in the middle (figure 5.13).

If, as seems very likely, this relationship is true for chitin fibers in cuticle, then only infinitely long fibers can be strained to the strain of the composite. Shorter fibers are strained by a lesser amount depending on the shear strain that is developed in the matrix as it transmits the stress to the fiber. This shear strain is measured as part of the overall strain of the composite and represents the difference between the total strain and the strain on the fibers. Ker estimates that in locust tendon a quarter of the total strain is due to shear in the matrix. This sort of deformation contributes to creep and energy losses if the bonds are relatively labile (e.g., hydrogen bonds rather

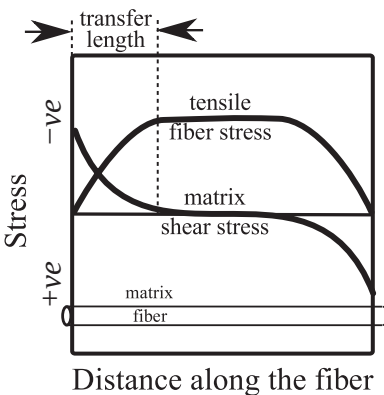


Figure 5.13. Standard composite theory suggests that the fiber, to be a reinforcement, has to be long enough for the forces to be transferred into it by shear between the fiber and the surrounding matrix.

than covalent bonds), which seems to be the case with locust tendon. Thus it appears that the stress–strain properties of fiber and matrix can be represented separately, as in figure 5.14, which shows the matrix to be elastic up to a yield point. The behavior after the yield point may be viscoelastic or plastic. Whether the fibers break or flow with the matrix depends on the shear stiffness of the matrix and the efficiency of transfer of the stresses to the fiber. The fiber will not break if the matrix cannot transmit sufficient stress to it, either because the matrix is too pliant or because the fiber is too short. Under these circumstances the fiber is unlikely to contribute much to the stiffness of the material, and the stiffness observed will be mainly that of the matrix (at small strains).

One other factor that is important if the fiber is to stiffen the matrix (still assuming that the fibers are all oriented along the direction of extension) is that

$$\sigma_c = \sigma_f V_f + \sigma'_m (1 - V_f) > \sigma_m, \quad [\text{Eq. 5.6}]$$

where σ_m is the strength of the matrix; subscripts c , f , and m represent composite, fiber, and matrix, respectively; and σ'_m is the stress in the matrix when the fibers fracture. This is the equation for line A in figure 5.15. V_{crit} (figure 5.15) can be rewritten as

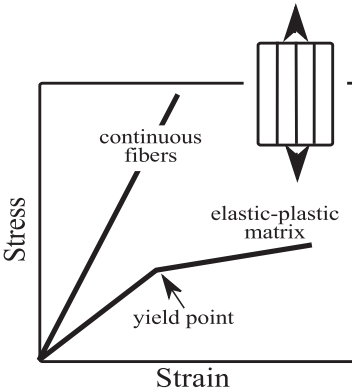


Figure 5.14. If the fibers are not continuous, the matrix becomes much more important in transferring forces.

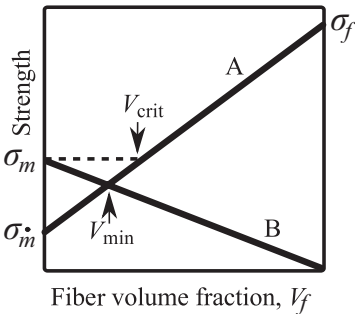


Figure 5.15. The strength of a composite is related to the geometry and properties of fiber and matrix, as explained in the text.

$$V_{\text{crit}} = \frac{\sigma_m - \sigma'_m}{\sigma_f - \sigma'_m}, \quad [\text{Eq. 5.7}]$$

If the volume fraction of the fibers falls below V_{crit} , then the composite will fail when the fibers break, leaving only the matrix to take the load. The strength will then be given by

$$\sigma_c = \sigma_f V_f + \sigma'_m (1 - V_f) > \sigma_m (1 - V_f). \quad [\text{Eq. 5.8}]$$

Thus there is a minimum volume fraction (V_{min}) of fibers that must be exceeded if the strength of the composite is to be given by an equation analogous to Eq. 5.6 (without the inequality):

$$V_{\text{min}} = \frac{\sigma_m - \sigma'_m}{\sigma_f + \sigma_m - \sigma'_m}, \quad [\text{Eq. 5.9}]$$

which is also shown in figure 5.15. If we take, from Ker, values of 4 GPa for the strength of the chitin fiber, 7.5 MPa for the stress in the matrix at the breaking strain of the chitin, and a fracture strength of the matrix 50 times higher than this (a generous estimate), then V_{min} is about 8.5% and V_{crit} about 10%. Because the chitin content of the tendon is at least 17% according to Ker, then it is clear that the chitin, not the matrix, absorbs the loads. Very few cuticles have the chitin oriented in a unique (preferred) direction: among the stiff cuticles only those that transmit force in a single direction (as locust tendon) show such preferred orientation. In most cuticles the chitin is oriented in many directions, as in plywood.

The orientation of chitin around the holes (pore canals, hair and bristle insertions) and other discontinuities varies in a rational manner; it carries the stress trajectories around the obstacle and so avoids stress concentrations. The effect of changing the orientation of the chitin is to lower the modulus of the composite to 3/8 for full isotropy in the plane, as opposed to the extreme anisotropy of stiffness in the tendon, which amounts to 530:1. Although the toughness of a fiber sheet with multiple fiber orientations has not been measured or compared with the fracture energy of something like tendon, which fractures much more cleanly across the chitin fibrils, it is likely that a difference of at least an order of magnitude in the fracture energy is gained.

5.3 To Stiffen the Matrix

The importance of the shear stiffness of the matrix has been emphasized. In insect cuticle the origin of this stiffness is obscure and a subject of some dispute. The problem arises because the matrix is produced as a protein in solution that has to be stiffened by the introduction of cross-links. The classical story (Pryor 1940) is that dihydroxyphenols are secreted into the cuticle, where they are oxidized to quinones and cross-link adjacent protein chains (figure 5.16). However, very shortly after Pryor's proposal, another was published in the same journal (Fraenkel and Rudall 1940), which advanced the hypothesis that the most dramatic event that occurs in the cuticle

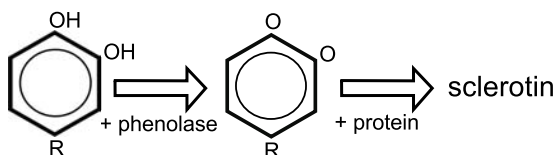


Figure 5.16. The basic chemistry of phenolic tanning—the conventional story.

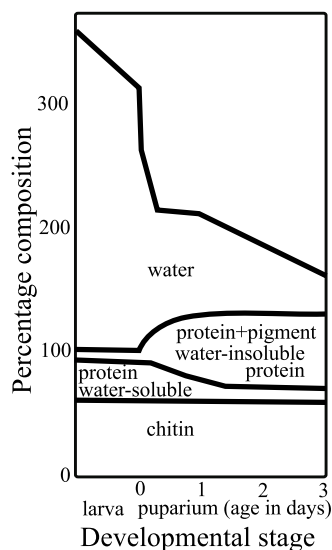


Figure 5.17. In many insect cuticles (this is a fly larva) the main change at tanning is a drop in water content (Fraenkel and Rudall 1940).

when it tans is the loss of water (figure 5.17) and that this is the most important factor. An important difference between the two models is that Pryor worked with the egg case of the cockroach, whereas Fraenkel and Rudall worked with the cuticle of a fly larva. The cockroach egg case has a number of advantages as a model system: it contains no chitin, only protein, so that any stiffening must be due to cross-linking of the protein and not to chitin–chitin or chitin–protein links; it is produced from time to time throughout adult life from a pair of glands (the colleterial glands) whose contents can be analyzed, allowing the precursors of the tanned protein matrix to be investigated; it is tanned as it is produced, so the process can be followed in a single egg case. By contrast, the cuticle of the maggot contains chitin; the processes within it must be inferred from what can be extracted from the cuticle, which is difficult if the processes being investigated are stabilizing the components and rendering them inaccessible. However, Pryor’s model has one highly significant advantage. The phenols involved are fairly reactive, so elegant biochemistry was possible. By contrast, experiments on the control by a cell of the water content of its surroundings were, and are, difficult to perform. It is probable that a major factor in the almost universal acceptance of Pryor’s model is that it is easy to manipulate. But the basic problem is that in all cuticles investigated the two processes of phenolic tanning and dehydration occur together: it’s a classic case of having two variables (at least) in a single experiment. Can a mechanical analysis supply the second experiment?

The most widely used of the experiments purporting to show that covalent cross-links exist in insect cuticle was insolubility of the cuticle proteins in any solvent less disruptive than 1 N NaOH at 100°C. What was never explained by those who used this criterion is how the chitin, itself “only” hydrogen-bonded (Minke and Blackwell 1978), could resist this treatment. Clearly, experiments that use a series of solvents to distinguish bond types do not take into account any cooperative effects that can

effectively shield bonds from the action of solvents. Additionally, as well as removing proteins, 1,2-diaminoethane (a breaker of H-bonds) removes a considerable proportion of the brown color from tanned cuticles, which suggests that a large proportion of the “tanning” agent is not covalently linked to the proteins (Hillerton and Vincent 1979). The results from extracting cuticles with various solvents raised doubts as to how much of the cuticular matrix is stabilized by covalent cross-links; insolubility in a mild reagent is not evidence for covalent cross-linking, since the bonds may simply be hidden within a cooperative structure that is “only” H-bonded. The global bond energy is important for stiffness, and a few H-bonds are a mechanical match for a covalent bond. Even more destructive of the theory of covalent cross-linking is that when sclerotized cuticle from the metathoracic femur of *Locusta* is swollen in an effective protein solvator, such as pure formic acid, it swells reversibly without much extraction of protein (Andersen 1981), and the swollen cuticular pieces have long-range elasticity, which indicates that the protein chains have considerable kinetic freedom. In cross-linking macromolecules, the state of solvation or swelling obtained when any cross-links were formed dictates the degree of swelling that can be obtained when the material is at equilibrium with a swelling medium (Treloar 1975). Therefore, if a material is cross-linked when it is swollen, as biochemists say must happen when the soft hydrated cuticle begins to be sclerotized, those cross-links cannot restrict the entry of free solvent at a later time, let alone cause solvent to be expelled. Therefore, it is not the phenolic cross-links that restrict the entry of water but secondary, noncovalent, links formed when the water between the proteins is removed. Thus it is not possible for covalent cross-linking on its own to provide the stiffness observed. If the water is removed under controlled conditions from maggot cuticle, there is a transition in stiffness of the type characterized by Nissan as typifying H-bonded materials (Vincent 2004, 2009). One of the main clues to possible action of the phenols comes from food science—the bitter drying taste of strong tea and cheap red wines that have been “overoaked” and have absorbed polymeric phenol from oak chips or the wooden walls of the storage barrels. These polyphenols are largely responsible for the mouthfeel, since they react with basic proline-rich proteins in the saliva. Astringency arises from precipitation of polyphenol–peptide complexes, which is an important protective mechanism in animals that consume polyphenols. The polyphenolic rings stack onto planar hydrophobic surfaces stabilized by cooperative binding, and the peptides become increasingly coated with polyphenols and eventually precipitate (Charlton et al. 2002; Guinard and Mazzucchelli 1996). This mechanism appears to be totally compatible with the way structural proteins react when treated with phenolics that then polymerize.

What is the nature of the “sclerotized” matrix? Presumably the structure will depend on a number of factors. If the proteins are more hydrophobic (figure 5.18), they will tend to be globular in an aqueous environment, and there is reason for thinking this is the case in locust tendon. The result may be a phased structure with strong secondary bonds within the globules and cross-links of some sort between the globules. But not all tanned cuticles have a high hydrophobicity index. The cuticle of *Calliphora* larvae is an example. Thus it should have a high water content and very little interaction between the protein chains, and probably does in the untanned state. But

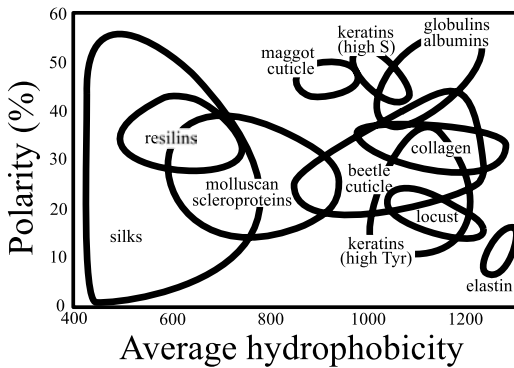


Figure 5.18. In terms of potential for binding water, materials destined to be tanned are very varied. The mechanisms for tanning may be equally varied (Hillerton and Vincent 1983).

just like silk, which also has a low hydrophobicity index, the protein of *Calliphora* larval cuticle is capable of forming β -structures. If such cuticle is extracted into 7 M urea, the extract dialyzed against water, and the dialyzed extract dried and examined in the infrared, it is found to be largely β -sheet (Hillerton and Vincent 1979). The protein in this conformation is largely insoluble. Thus it is possible that in some cuticles, dehydration causes the formation of crystalline structures in the matrix, which then becomes as insoluble as chitin, and for much the same reasons. This mechanism was originally proposed by Fraenkel and Rudall (1947).

In conclusion, it should be pointed out that if the causes and mechanisms of sclerotization are still uncertain, it is partly because there seems to be no single set of circumstances for producing a stiff cuticle. It is to be expected that if the aim is to introduce numbers of primary and/or secondary cross-links, there will be many ways of doing so.

5.3.1 OTHER SCLEROTIZED SYSTEMS

A number of other tanned protein systems exist. These are typically brown, stiff, and phenolic and often contain chitin. The hard brown sheath around *Obelia* (Coelenterata, Hydrozoa) has been shown to be made of a protein–chitin material (Knight 1968): phenol precursor (catecholamine), phenoloxidase, and protein are found in vacuoles in tanning cells, which are especially numerous in growth areas. The egg shells of many turbellarians, trematodes, nematodes, and some cestodes are tanned protein, as are the chitin-containing hairs of many annelids, the tubes of *Sabellaria*, and the like. One of the nicest examples of the importance of hydration in a tanned system is the beak of the Humboldt squid, *Dosidicus gigas*, which is made from protein and chitin, just like insect cuticle, and is layered. In the hardened tip of the beak about 15% of the dry weight is contributed by catechol cross-links (Rubin, Miserez, and Waite 2010). As figure 5.19 shows, water content is by far the most important factor in controlling stiffness. But we still can't decide whether the “cross-links” are directly responsible for the increased stiffness or whether they drive the water out chemically and form stable interactions, which may have more to do with retaining the waterproofing agent than with reducing the mobility of the proteins. The outer covering of the shells of many bivalves (the periostracum) such as *Mytilus* is made of tanned protein and may

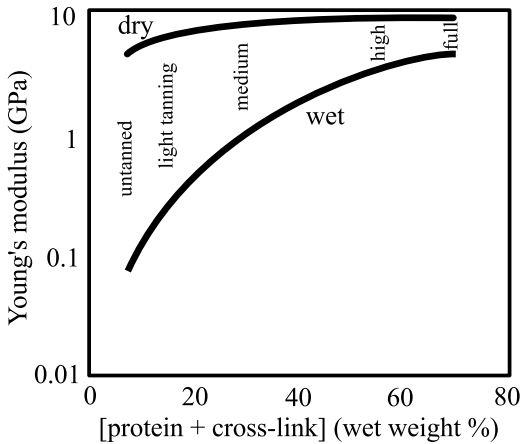


Figure 5.19. Comparison of water content and tanning in stiffening the beak of the Humboldt squid, *Dosidicus gigas* (Rubin et al. 2010).

contain chitin. The byssus threads of *Mytilus* are phenolically tanned, as is the glue that holds them onto the rock. Again, the analysis recognizes that water is an important component in the system. The glue of the mussel is a peptide containing DOPA.

Water is still the biggest problem. Adhesives technologists go to great lengths to exclude water, both during the creation and setting of a joint and during its useful lifetime. However, there is a general principle of solving problems: if a component of the problem system is identified as causing the main upset, then it is strategically better to incorporate that component into the system than to reject it. This strategy ensures not only that the problem disappears but that it will never reappear as a threat to the system. This suggests that any successful solution to the problem of underwater adhesion should include both water and the bacterial film as positive parts of the strategy. In turn this suggests the form of a successful model system, and the sort of system that is likely to be found in nature, and therefore the search image which the researcher should adopt. Few of the suggested mechanisms for byssal adhesion seem to take this approach, which may be a mistake. In a different system—cyanoacrylate adhesive—a small amount of water, garnered from the surfaces to be glued, is an essential part of the chemistry. This interpretation of the problem has been realized by Messersmith and his colleagues (Lee, Scherer, and Messersmith 2006), who found that strong bonds are formed between DOPA and organic and inorganic surfaces in the presence of water, which is accepted as a crucial characteristic for a protein adhesive operating in the wet marine environment.

Ultimately it's necessary to recognize that dehydration is probably only one of the processes that sclerotized systems can deploy. Others include the addition of the transition metals Zn, Mn, Fe, and Cu. All these have been reviewed extensively (Rubin, Miserez, and Waite 2010).

5.4 Hardness and Indentation

Bryan and Gibbs (1979) were the first to report zinc in the jaws of a worm (*Nereis diversicolor*). The authors were originally interested in pollution by heavy metals, but

when they realized that 20% of the entire zinc burden of the worm was in the jaws, and 40% in *N. virens*, and that the zinc content was largely independent of environmental concentrations of zinc, they thought it might be structural. Silica and calcium salts are obvious candidates for providing local hardening, and the presence of iron (as goethite) in molluscan radular teeth has been known for more than 100 years (Sollas 1907) and was examined more closely by Runham et al. (1969), but the finding of copper (Gibbs and Bryan 1980) and manganese (Robertson, Hillerton, and Vincent 1984) was novel.

Zinc and manganese have been found in a number of other animals, nearly always associated with the mandibles. In nearly all herbivorous insects (but not the carnivorous ones) that have been examined one of these metals is present in the cutting edge of the mandibles (Hillerton, Reynolds, and Vincent 1982; Hillerton and Vincent 1982), where it is associated with increased hardness. The metal is present in significant amounts—up to 10% of the dry weight of the whole mandible. The mandibles of the locust have a hard capping with a hardness of about 35 kg mm^{-2} . The capping is on the inner edge of one mandible and the outer edge of the other. The soft faces (hardness about 18 kg mm^{-2}) work against each other rather like a pair of scissors, so that they wear each other away and continually break away the blunt edge by undermining (Hillerton 1980), much like a hobby knife with a blade whose tip, when blunted, can be broken off to expose a fresh, sharp point. Some spiders and scorpions can have zinc, manganese, or iron; indeed, the arachnids are different from other invertebrates investigated in that all three elements can be found in different areas of the mouthparts in a single individual (Schofield and Lefevre 1989).

The chemistry of incorporation of these metals is uncertain. The teeth of the chaetognath worm *Sagitta* are pure chitin. On the outer surface they have a light brown layer and significant amounts of zinc (Bone, Ryan, and Pulsford 1983), so protein is not an essential part of the system, but phenolic residues might be. This evidence ties in with observations on the timing of incorporation of zinc and tanning agents in insects and spiders (Schofield et al. 2003). There is plentiful literature on the chelation of metals by catechols and other dihydroxy phenols (Andjelkovic et al. 2006; Hider et al. 2001; Le Nest et al. 2004a, 2004b), some of which are biogenic (Ragan, Smidsrod, and Larsen 1979; Wang, Jónsdóttir, and Ólafsdóttir 2009). Melanin (a phenol derivative) has also been implicated in biological metal chelation and the generation of extreme mechanical performance (Moses et al. 2006; Riley 1997). Indeed, the jaws of *Glycera* have been shown to perform mechanically as well as many advanced man-made materials (figure 5.20).

The test used to measure hardness is indentation (see section 6.2.2 for more analytical detail). For instance, the Vickers hardness test involves pressing a pointed diamond, cut to a four-sided pyramid shape and of standard size, into the material under test. Because the diamond probe is tapered, the size of the indentation it leaves in the material under test is proportional to the depth to which it has been pressed, so simple measurement of the length of a diagonal of the hole gives a measure of the hardness. As the diamond pyramid sinks into the material, it causes shear deformation. Thus the hardness measured is closely related to shear stiffness. But the measurement that is made is the amount of deformation remaining after the deforming force has been removed, so the shear strength of the material is also important. After the deforming force has been removed, the material is free to recover. Thus the ultimate shear strain

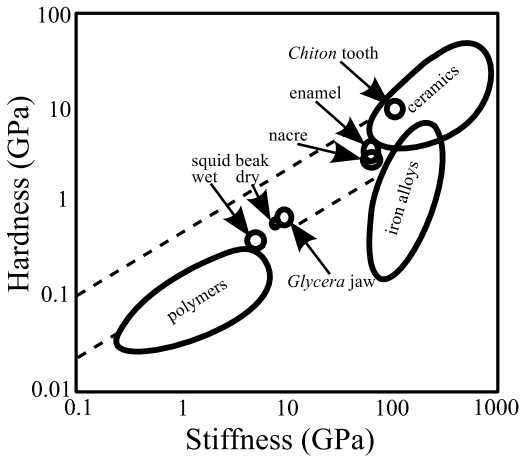


Figure 5.20. Hardness of some biological materials, either made of ceramic, hardened with Fe^{3+} , Cu^{2+} , or simply sclerotized (Rubin et al. 2010).

of the material is also significant, since it influences the recoverable shear strain. Viscoelastic properties affect the hardness as well, because the more viscoelastic the material is, the longer it will go on recovering after the load is removed. The time at which the hardness measurement is made will be important. Hardness, then, is really a relative term when applied to viscoelastic materials. It is expressed in units of stress that are then equated directly with shear strength.

5.5 Cellular Materials

Plants, many parts of animals, bits of aircraft flying surfaces, helicopter rotor blades, skis, wasp nests, and the doors on the underground trains in Hong Kong all have one thing in common: they are “skin–core,” or sandwich, structures. A familiar one is the bracket fungus, or polypore. The outer covering is a relatively stiff tensile membrane, but the inner material is very light and foamlike, made of cells. This arrangement has a number of structural advantages as well as the obvious biological ones. A few simple experiments bending the polypore fungus will convince you that the outer membrane resists most of the stress. If you damage the skin, the cellular core fractures in tension relatively easily, although compressive failure is more controlled, depending on how dry the fungus is. Gibson and Ashby (1997) have regularized much of the information about cellular materials, starting from models much like that shown in figure 5.21, which is an open-cell foam made from struts each of length l and width and thickness both t . When the foam is compressed, the various components act as columns or beams. If the columns do not buckle out of the way, then most of the deformation is due to bending of the beams, and the foam can be considered as a large collection of small beams. Standard beam theory (Timoshenko 1936) gives the deflection of a beam of length l loaded at its midpoint by a force F as proportional to

$$\frac{Fl^3}{E_s I}, \quad [\text{Eq. 5.10}]$$

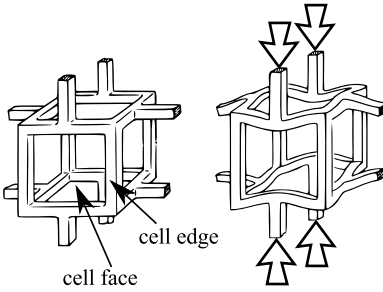


Figure 5.21. A simple open cell being squashed (Gibson and Ashby 1997).

where E_s is the stiffness of the material used to make the beam and I is the second moment of area of the beam, which in its turn is proportional to the fourth power of the thickness, t , of the beam. The relative density of the foam is defined as the density of the foam, ρ^* , divided by the density of the beam material, ρ_s . This ratio is proportional to $(t/l)^2$.

The force, F , is related to the compressive stress and is proportional to σl^2 , and the strain for a given displacement is inversely proportional to l . Young's modulus of the foam, E^* , becomes

$$E^* = \frac{\sigma}{\epsilon} = \frac{C_1 E_s I}{l^4}, \quad [\text{Eq. 5.11}]$$

where C_1 is a rather convenient constant. From the preceding argument we can now deduce that

$$\frac{E^*}{E_s} = C_1 \left(\frac{\rho^*}{\rho_s} \right), \quad [\text{Eq. 5.12}]$$

so a log–log plot of relative stiffness against relative density has a slope of 2, which gives a convenient way of checking the theory and seems to be true for a wide range of materials and foam shapes. However, this theory describes only the elastic part of the compressive behavior, that is, at small strains. At larger strains all sorts of nasty things start to happen: the columns buckle, and the beams bend plastically or break in a brittle manner. Eventually, the bits get squashed so close together that they press directly on one another, and the modulus starts to rise rapidly toward the stiffness of the solid material. This phase is known as *densification*. The precise behavior in the zone between elastic behavior and densification depends on whether the foam material is rubbery, elastic–plastic or brittle. Figures 5.22 to 5.24 show the behavior of three such materials in compression. In tension there is little qualitative difference between the overall shapes of the curves of a cellular and a noncellular solid.

In the animal world the most-studied cellular materials are ceramic (bones of one sort or another), which we will deal with in the next chapter. But there are some keratinous cellular structures. The rachis of feathers is filled with a cellular foam, which increases its stiffness by 16% (Purslow and Vincent 1978), as are porcupine quills (Vincent and Owers 1986) and the spines of hedgehogs (*Erinaceus* sp.). A theoretical

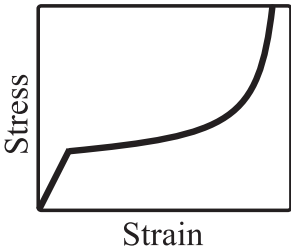


Figure 5.22. An elastic cellular material being squashed (Gibson and Ashby 1997).

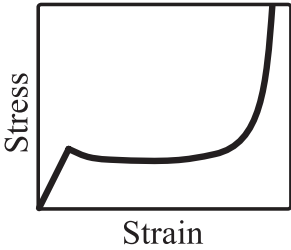


Figure 5.23. A yielding cellular material being squashed (Gibson and Ashby 1997).

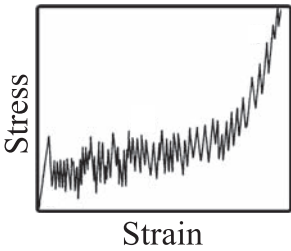


Figure 5.24. A brittle cellular material being squashed (Gibson and Ashby 1997).

analysis shows that filling a tube with a uniform honeycomb or foam filling is much more efficient than adding extra material to the wall of the tube. In addition to having improved resistance to buckling, a shell with a compliant core is much less sensitive to imperfections (Karam and Gibson 1995). These authors showed that hedgehog spines, in particular, are extremely efficient structures in their ability to resist bending (figure 5.25) (Karam and Gibson 1994). In the hedgehog the longitudinal stringers (figure 5.26) provide support for the wall, so that the spine can buckle elastically in the Euler mode while storing the maximum amount of strain energy. This characteristic may account for the numerous reports of hedgehogs' being able to bounce and the fact that they can have a semiarboreal lifestyle without being able to fly or brachiate. Hedgehogs do not exist in the Americas, but their niche may partly be occupied by the tree porcupine, *Coendou*, which is unusual among the porcupines, but similar to hedgehogs, in that all its spines are the same length. Perhaps it, too, can bounce?

Cellular materials come into their own in the plant world, where the success of the design is very apparent. The most studied of these materials, mainly because of its commercial importance, is wood.

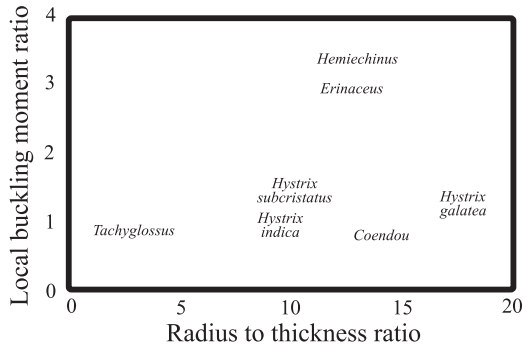


Figure 5.25. Quills of hedgehogs and porcupines resisting buckling, considering their size. The hedgehogs outperform the porcupines quite easily, which suggests that their spines have evolved for different uses (Karam and Gibson 1994).

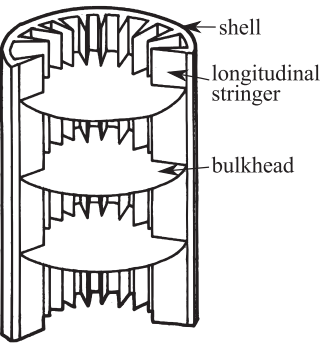


Figure 5.26. A sketch of the structure of a hedgehog spine (about 1 mm diameter) (Vincent and Owers 1986).

5.5.1 WOOD

The specific stiffness of wood (stiffness per unit weight) is as good as that of steel. Moreover, steel costs about sixty times more than wood per ton. Along the grain, wood is a quarter as strong as mild steel, so its specific strength is about four times that of steel. Thus its commercial importance is great—if only we could stop it from rotting. But, unfortunately, the techniques used to stop the rot very often reduce the strength of wood. Also because of its complexity, wood is not always an easy material to deal with. It needs a much more skilled and sympathetic approach than do metals (Gordon 1976). In tension, and to a first approximation, the cells can be considered as a system of parallel discontinuous fibers embedded in a matrix. This model does not take into account the fine morphology of the cells, but in practice this seems not to matter. For instance, the mean axial tensile stiffness and strength of single cells of Sitka spruce (*Picea sitchensis*) are 12.5 GPa and 170 MPa, respectively (referred to the gross cross-sectional area). These values can be used in formulas like those for fibrous composites (Eqs. 5.1 and 5.6) if we neglect the contribution of the matrix:

$$E_c = E_f V_f; \quad [\text{Eq. 5.13}]$$

$$\sigma_c = \sigma_f V_f. \quad [\text{Eq. 5.14}]$$

The volume fraction of cells in Sitka spruce is about 0.8, so the calculated values for E_c and σ_c are 10 GPa and 136 MPa, respectively, which agree well with the mean experimental values of 10 GPa and 100 MPa. To some extent these results might imply that the cells are to be considered as separate entities from the mechanical point of view. In an exhaustive study of the mechanics of wood, Mark (1967) showed that this is so and that a single wood cell behaves differently than the same cell cut in half longitudinally, which emphasizes the importance of the cellulose and its continuity for the mechanics of the cell. Alternatively, one could consider the cellulose microfibril as the basic unit of the composite and derive the mechanical properties from the information available on the laminated structure of the cell wall. This is a complex problem, but Mark has given a comprehensive account of this approach, as has Jeronimidis (1980).

In tension, wood is not only stiff but strong and very tough. These properties were very upsetting to materials scientists, since they could not account for them. The only workable idea was to model wood as a fibrous composite material with the wood cells as the fibers (Dinwoodie 1968). The main mechanism available for toughening such materials is “fiber pullout.” When the composite breaks, if the fibers are not stuck too strongly to the matrix, the crack can travel up and down the fibers, loosening them from the matrix and causing them to break some distance away from the main crack surface. As the two halves of the piece of material are pulled apart, the fibers are pulled out of their holes and lose energy as friction. It is certainly easy to see fibers pulled out from wood when it breaks—the wood cells appear as splinters—although one does not see many ends of the cells. The cell walls are torn apart. But mathematical modeling showed that a fiber pullout mechanism cannot use up enough energy—it uses only a tenth of that actually measured. Wood must be doing something more subtle.

The softwood cell has been drawn and redrawn many times—and here it is again (figure 5.27). The cell is composed of a number of layers with various arrangements of cellulose microfibrils stuck together with lignin and is attached to its neighbors by another lignin-rich layer—the middle lamella. The thickest wall, about 80% of the thickness in most woods, is the S2 layer. In it the cellulose is oriented in a single direction, winding around the cell at an angle averaging about 15° to the longitudinal axis (figure 5.28). This feature is responsible for the high work of fracture of wood (Jeronimidis 1978). You can easily see the mechanism for yourself by taking a helically wound paper straw (preferably at least 10 cm long and 3–5 mm in diameter) and pulling carefully on the ends. When the straw breaks, it does so partly by buckling inward and partly by developing a helical fracture that runs some distance along the straw (figure 7.4). This type of failure has been observed in wood (figure 7.6) where the individual cells additionally pull apart laterally as they buckle inward, generating up to 200 times the nominal surface area of the fracture and thus absorbing even more energy. The orientation of fibers in the S2 wall is something of a compromise

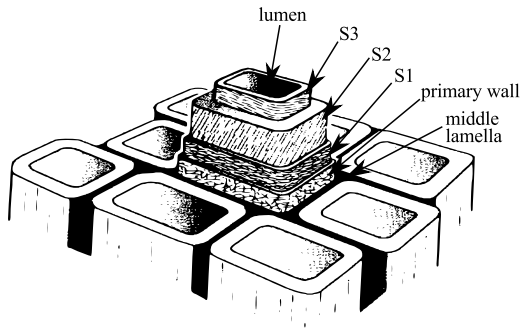


Figure 5.27. A section of softwood (conifer), showing the basic structure of the cell wall.

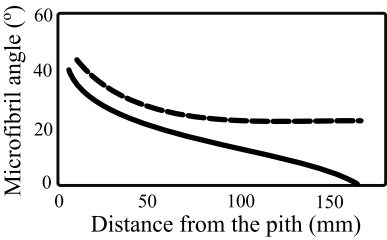


Figure 5.28. Angle made by the cellulose microfibrils in the wall of wood cells depending on position within the trunk.

between stiffness and toughness. If the fibers were oriented parallel to the cell axis (as happens in grasses), the cell would be stiffer in tension but could not take advantage of the buckling mode of failure and so would be less tough. This is not so important for an annual plant, which is not exposed to the same sort of external loads as a tree. But if the fibers were oriented at right angles to the axis of the cell, the toughness would be increased but the axial stiffness reduced. If the compromise is well calculated, it is possible to increase the toughness of artificial composites using the same morphology—though on a much larger scale—by a factor of 10 or more over that of conventional glass–fiber–resin composites at the expense of only a moderate decrease in axial stiffness (see chapter 7). When the two halves of the piece of wood finally part company, the spiral fractures leave sharp-ended splinters.

In compression, the cells of wood are unstable and buckle locally, so the compressive strength of wood is less than the tensile strength. The buckles appear as lines across the wood (compression creases or kink bands) and can easily be produced in a piece of 6-mm-diameter dowel in four-point bending, in which the central length is subjected to an even stress along its length, so many compression creases can be formed. With a bit of experience it is possible to feel the plastic deformation involved with creasing. The effect of the creases is most easily shown by turning the dowel around and bending it again with the compression creases on the tensile surface. The creases are then the equivalent of cracks and the focus for stress, so the dowel breaks at these points. The wood does not produce splinters, which shows that the wood cells were broken. Compression creases are quite safe, so long as they are on

the compression side. In fact, they protect the wood from overloading by absorbing energy. Obviously, it is important that they remain on the compression side—if the stress is reversed, the object will break. For instance, never slow down a rowing boat by allowing the water to flow against the back of the blade; reverse the blade so that the water pushes against the spoon face, and the loom (the shaft) of the oar is loaded in the normal manner.

5.5.2 SET A CRACK TO CATCH A CRACK

The handles of tools such as axes and hammers, parts of wheels (notably the spokes), and the chassis of vehicles and carriages are traditionally made of ash or hickory. These two woods are able to store elastic strain energy and absorb shocks. This property seems to be made possible by the presence of holes. Hardwoods (from broad-leaved trees, mostly deciduous in temperate climates) are immediately distinguishable from softwoods (from needle-bearing trees, mostly evergreen) by the more complex morphology of their wood. One characteristic is the presence of large vessels in the xylem, which provide the main route for transport of water. These holes provide localized weak areas of low density around which the wood collapses preferentially. If the vessels are arranged in layers (which happens in some oaks and willow), then the wood will crack fairly easily, since the holes represent a weak layer within the material. But if the vessels are arranged uniformly throughout the wood (as opposed to “randomly,” which allows clumping), then the damage is spread evenly, and the full toughness of the wood can be realized (Hepworth et al. 2002). That a hole weakens a material should not be surprising, but that a hole can be instrumental in providing toughness is somewhat counterintuitive. This structure was shown to be an important consideration by Jim Gordon (Cooke and Gordon 1964): a crack tip does not have a uniform strain field ahead of it—there is an induced stress of about a fifth of the applied stress that acts along the line of the crack. This stress tends to open up weak areas ahead of the main crack, so that the advancing crack will be blunted as it moves into a ready-made hole (figure 5.29). Such interfaces can be incorporated into the design of the material as sacrificial components, but in hardwoods the holes are already there.

In 1921 Griffith published a paper in which he showed that cracks start from stress concentrations and propagate by feeding on strain energy. But what happens if there are many stress concentrations? We have psychotic starter cracks! How do we choose from among them? As each crack grows, it removes strain energy from the surrounding region and so inhibits the further propagation of other starter cracks. However, if

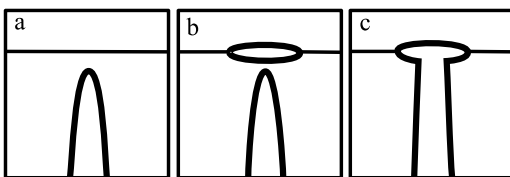


Figure 5.29. The Cook-Gordon crack-stopping mechanism (Cooke and Gordon 1964).

there are too many holes in the structure, it will be weakened, since the cracks have only to connect the holes for the material to break. Thus we find that hardwoods with evenly distributed vessels are tougher in impact than would be predicted from the toughness of softwoods, whose cells are all more or less the same size as one another.

Knowledge of the properties of different woods has always been incorporated in the way we use them, especially early in our history, when wood was of major importance as a structural material. Ash, one of the woods with evenly distributed holes, which benefits from the toughening mechanism described in the preceding paragraph, is virtually unknown in the handles of prehistoric “axes,” which suggests that the axe was not used in the way we use it today, which requires the handle to absorb impact energy. Perhaps the axe head was used as a wedge, to be hit with another tool. The axe handle enabled one to manipulate the wedge without the danger of hitting the fingers. Ash is also used for making spears. Other shock-absorbing woods include rock maple, box, beech, birch (for arrows), hornbeam, putaputaweta, hinau (spears, palisades), mangeao (barrels, wheels, handles), apple (mallets, cogs), and rata and pernambuco (railway sleepers). The latter wood is highly prized for making violin bows, but since it is now more or less extinct, the main source today is superannuated railway sleepers from Brazil.

Biological Ceramics

The trouble with using protein to make a skeleton is that it is metabolically expensive. Insect skeletons are made of protein because it is relatively light, and with chitin as the fiber, they are also stiff and tough. Moreover, the mechanical properties of cuticle are very closely tailored to its use by variations in the properties of the protein matrix, so that insect cuticle is adapted to many forms, from hard mandibles to elastic and extensible membranes. Protein is thus eminently suitable for the skeleton of an animal that owes a large part of its success to its capacity for flight. But protein has to be synthesized—an energy-consuming process—and is largely placed outside the metabolic pool by the necessity for mechanical stability. The more it is cross-linked, by primary or secondary bonds, the less soluble it becomes and the more difficult to recycle when the insect moults.

In Crustacea—arthropods that are mainly aquatic and do not fly—the amount of protein in the exoskeleton is reduced and replaced with calcium carbonate. The specific gravity of calcium salts is more than twice that of protein and chitin, but since this difference partly reflects closer packing of the molecules (and hence greater bond density and greater stiffness, E , of 100 GPa), it is not patently obvious why the calcium-containing exoskeleton should be heavier. A likely explanation is that it is much more brittle and so under a given force, a thicker skeleton will be far less likely to approach its ultimate strain because the stress will be that much less. The insect can control the deformability of its skeletal material much more closely, but the (mainly) calcite of decapods is brittle because it is crystalline, and this property cannot be changed, so its bulk fracture properties can be circumvented only by careful design of the material.

How can animals and plants circumvent the low fracture toughness (and hence general durability) of ceramic materials to make proper use of their greater stiffness and strength? One particularly elegant example is found in the calcified cuticle of one of the forelimbs of a stomatopod crustacean, *Gonodactylus chiragra* (Currey, Nash, and Bonfield 1982). The limb, powered by a catapult system, is used to break mollusc shells. The outer layers reach a hardness of 10 GPa, about 30 times the surface hardness of a crab claw (Zhou et al. 2010) and far greater than most of the values quoted in table 6.1. The explanation is that phosphate is included in the normal calcite of the exoskeleton, though (as Currey points out) its effect might be reinforced because the sample had been kept in alcohol for a number of years, which would tend to remove water and hence decrease the capacity for plastic deformation and resistance to cracking. Even so, the performance is impressive.

TABLE 6.1
Comparison of calcium-containing minerals in animals

<i>Chemical formula</i>	<i>Common name</i>	<i>Occurrence</i>	<i>Specific gravity</i>	<i>Hardness (GPa)</i>
CaCO ₃	Calcite	Birds' eggs, echinoderm test, teeth, octocorallian spicules, sponge spicules, some brachiopods, crustacean exoskeleton, mollusc shells	2.71	1.6
	Aragonite	Some reptile eggs, some foraminifers, many mollusc shells	2.93	2.3–3
CaMg(CO ₃) ₂	Dolomite	Echinoderm teeth	2.85	2.3–3
MgCO ₃	Magnesite	Sponge spicules	3.01	3
Ca ₃ (PO ₄) ₃ (OH)	Hydroxyapatite	Bone, teeth, dermal ossicles, young molluscs	3.1–3.2	5.4
SiO ₂ (H ₂ O) _n	“Amorphous” hydrated silica (opal?)	Sponge spicules Limpet radular teeth	2.0–2.2	6.7–9.8
CaF ₂	Fluorite		3.18	3
CaSiO ₃	Wollastonite		2.9	5.4
(Ca·Na)(Al,Si)AlSi ₂ O ₈	Plagioclase, scapolite		2.6–2.8	8
Ca ₂ Al(AlSi ₃ O ₁₀)(OH) ₂	Prehnite		2.9	9.8
CaAl ₂ Si ₂ O ₇ (OH ₂)·H ₂ O	Lawsonite		3.1	15.7

6.1 Calcium Salts or Silica?

One advantage of using calcium salts seems to be metabolic cheapness. Unfortunately, the metabolic cost to an animal of making something out of calcium salts has not been calculated. Certainly, calcium can be relatively easy to come by. Seawater, especially along the tropic strand, is saturated with calcium. Two major classes of calcium salts are found in skeletons—phosphates and carbonates. These salts have different crystalline forms, which introduce an additional factor in the variety of materials possible. In general, phosphates (as hydroxyapatite) occur in conjunction with collagen, and carbonates (as calcite, aragonite, etc.), in conjunction with other proteins and polysaccharides. Phosphates also tend to be the major form in vertebrates and brachiopods, whereas carbonates predominate in most other invertebrates. There are other salts, such as oxalates, but these are not common. Obviously, such salts are largely insoluble once formed and precipitated. Some of these salts are listed in

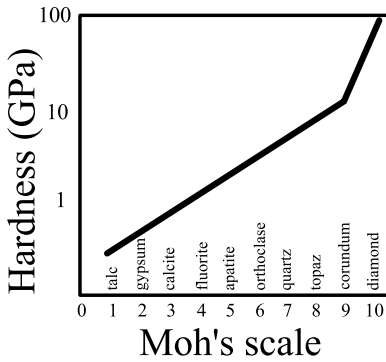


Figure 6.1. Relation between Moh's hardness, a scratch test, and hardness.

table 6.1 together with an indication of their distribution in the animal kingdom, their density, and their hardness (figure 6.1).

The smaller the ion, the denser is the packing and the denser are the bonds in the crystal. Hence, higher stresses can be borne. For the same charge the ions of manganese, iron, cobalt, nickel, magnesium, and beryllium are smaller—in that order—than calcium. Thus a calcium salt with any of these ions incorporated into it will be harder. Dolomite is incorporated into the teeth of echinoderms (Brear and Currey 1976), and iron is found in the hydroxyapatite of the teeth of some fish (Motta 1987).

Another way of expressing bond density is in terms of the density of the material (assuming the chemistry does not change); table 6.1 shows that the greater hardness of aragonite is due to the closer packing of its ions and that apatite is probably also more tightly packed within the crystal structure. This comparison of density and hardness also shows that the silicates, which are largely covalently bonded, are less dense than the ionic salts at equivalent hardness. Thus if hardness (or, more probably, strength) is at a premium, then silicates are the material of choice. But, then, why are silicates so rare? The answer probably has to do with the chemistry of silicon. It may be metabolically more expensive to incorporate silicates; certainly, silicates are, in general, less soluble than calcium salts. The cycle of silicon in nature is thus complicated by the almost complete removal of the assimilated element from the pool of available nutrients when the organism dies or is eaten. By contrast, some calcium salts (e.g., sulfate and chloride) are much more soluble than others (e.g., carbonate), so that Ca^{2+} is not only readily available but can be made insoluble when sequestered.

Ultimately, there may not necessarily be any advantage for skeleton or spicules to contain silica. A consideration of the strength of very fine ceramic fibers shows that size is just as important a factor and that the advantages to be gained in the extra strength of silica can easily be lost, as with glass fibers, through growth steps that can act as stress concentrators. These steps initiate cracks that cause the crystals to fail well below the theoretical strength (Gordon 1976). This problem seems to have been circumvented by a remarkable structure produced by members of the Hexactinellidae, or glass sponges. Of this group, the most studied for their mechanical and

structural properties are members of the genera *Monoraphis* and *Euplectella*; they produce silica skeletons made of rods of opal. As an example, *Monoraphis* is a rare sponge with a single anchoring spicule several millimeters in diameter and up to a meter long (Levi et al. 1989; Müller et al. 2008; Wang, Schröder, and Müller 2009). The spicule can be bent into a circle without breaking because it is made up of a central solid rod surrounded by 200 or more concentric layers varying from 10 μm thick near the center to 3 μm at the periphery. Thus each layer can be bent through a large angle without experiencing appreciable strain. A comparison of load deflection curves of a spicule and a silica rod of similar size (figure 6.2) in three-point bending shows that the spicule achieves sevenfold higher deflection because its layered structure can control the propagation of cracks (figure 6.3), which travel along the spicule and produce a cone-shaped fracture. The spicule is thus able to withstand a fourfold higher stress before failure and even then breaks in a relatively controlled fashion, which gives it a 30-fold greater work to fracture in tension. It achieves greater stiffness but lower strain in tension and breaks straight across, which indicates that the layers are equally loaded. Questions to be answered include the following: Why is the stiffness in bending and tension different? If stiffness is a material property, then there is a structural element that comes into play here. What might it be? Is there a selective advantage in evolutionary terms?

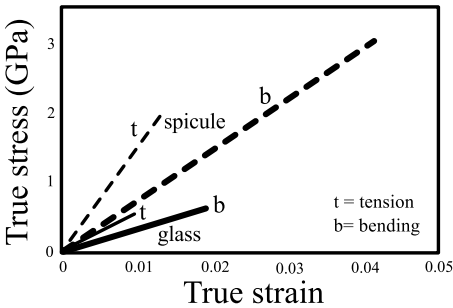


Figure 6.2. Spicule of *Euplectella* (dashed lines) and a glass rod (solid lines) of the same size compared in tension and bending (Walter et al. 2007).

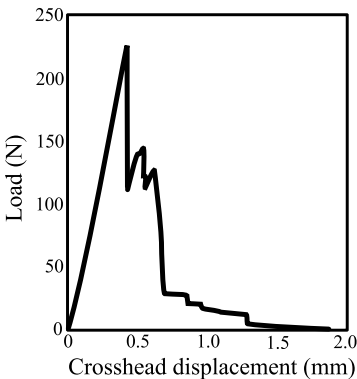


Figure 6.3. Bending failure of *Monoraphis* spicule, showing graded fracture, breaking a few layers at a time (Müller et al. 2008).

6.2 Problems with Mechanical Tests

Glass sponge spicules are unusual in that they are high-stiffness materials that form large uniform structures ideal for mechanical testing and modeling. Most stiff biological materials are much more complex, being hierarchical and structurally heterogeneous. It can therefore be difficult to isolate a specimen that is sufficiently uniform and well shaped to be amenable to “standard” mechanical tests (which basically fall into the categories of stretch, bend, or twist), and so we have to resort to less direct or less well established techniques.

6.2.1 PROBLEMS WITH SHEAR

Many tests to measure stiffness and strength are performed on a beam in three-point bending. This configuration is especially convenient, since loads can reasonably easily be applied to the specimen, compared with the complexities of fixing ends for compressive and tensile tests, or providing the extra length needed for four-point bending. However, if the beam is short relative to its depth, it will experience a significant amount of shear through its thickness, and the calculated Young’s modulus will be too low (figure 6.4). This difference can be accounted for by knowing enough about the material, but it is far more reliable to avoid this complication. Suitable span-to-depth (S/D) ratios, chosen to avoid shear, have been recommended for various materials including metals ($S/D > 8$), timber ($S/D > 24$) (Roark and Young 1975), and fiber-reinforced composites (Zweiben, Smith, and Wardle 1979), but none have been given for nacre and other biological materials. The importance of getting this ratio right is shown by experiments with a unidirectional Kevlar–polyester composite (Zweiben et al. 1979): using an S/D of 16 instead of the required 60 drops the measured modulus to about 65% of its true value! With nacre, an S/D ratio of at least 16 is found to be necessary (Jackson, Vincent, and Turner 1988). This problem has been approached more analytically by Spatz, O’Leary, and Vincent (1996).

In three-point bending, the deflection of a rectangular beam (Timoshenko and Goodier 1970) is

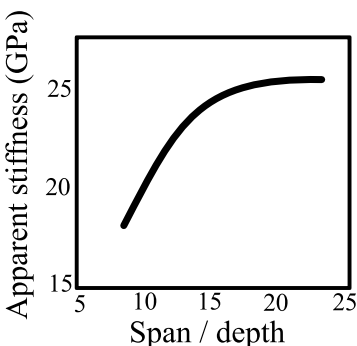


Figure 6.4. Stiffness of bone is underestimated if the depth of the beam is too great (Spatz et al. 1996).

$$\Delta x = \frac{Fs^2}{4E_{\infty}wd^3} + \frac{3}{10} \frac{Fs}{Gwd}, \quad [\text{Eq. 6.1}]$$

where F is the centrally applied force, s is the span, w is the width, d is the depth of the specimen, E_{∞} is the limiting Young's modulus measured on a beam with an infinitely large S/D ratio, and G is the shear modulus. The factor $3/10$ compensates for the nonuniform distribution of shear strain across the section of the beam. Jackson (1986) rearranged this equation to give

$$\frac{1}{E_{app}} = \frac{1}{E_{\infty}} + \frac{6}{5} \frac{1}{G} \frac{d^2}{s^2}, \quad [\text{Eq. 6.2}]$$

with the flexural modulus

$$E_{app} = \frac{F}{\Delta x} \frac{s^2}{4wd^3}. \quad [\text{Eq. 6.3}]$$

Thus we can estimate the apparent Young's modulus as a function of the S/D ratio (figure 6.5) and determine the S/D ratio at which the test will estimate the Young's modulus as measured in tension. In osteate bone measured in three-point bending and with the osteons oriented along the length of the beam, the minimum S/D ratio to reduce shear to near zero is 25. This approach also allows us to estimate the shear modulus.

6.2.2 INDENTATION

Hardness is important not so much as an intrinsic property (though it probably is important in abraded materials such as tooth enamel) but as a measure of the tensile strength of a crystal, which is one-third of its hardness but less if dislocations are free to move within the crystal and increase the amount of plastic deformation. Thus the hardness of a crystal can give an indication of the strength of the constituent material, which is very useful when the material is of a shape or size on which it is difficult to conduct direct tensile tests. The correlation is possible because hardness is a direct function of the energy of the interatomic bonds and the type of bonding. This relationship has been shown for a variety of minerals: in general, minerals whose atoms are bonded covalently, such as silica, aluminium oxides, and diamond, are very much harder than minerals whose atoms are bonded ionically, such as sodium chloride and calcium carbonate. Hardness is also affected by the symmetry of bonding—the plates of graphite are actually harder than diamond, but they can slip over one another and so form a soft material in bulk. Indentation can also give an estimate of the stiffness. For example Eq. 6.4 is for a spherical indenter of radius R penetrating with force P to a depth h a material of stiffness E and Poisson's ratio ν (Johnson 1985):

$$P = \frac{4\sqrt{R}}{3} \frac{E}{(1-\nu^2)} h^{3/2}. \quad [\text{Eq. 6.4}]$$

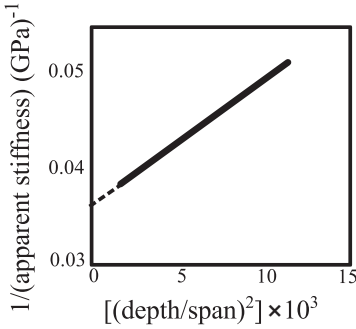


Figure 6.5. Data from figure 6.4 replotted according to Eq. 6.2 (Spatz et al. 1996).

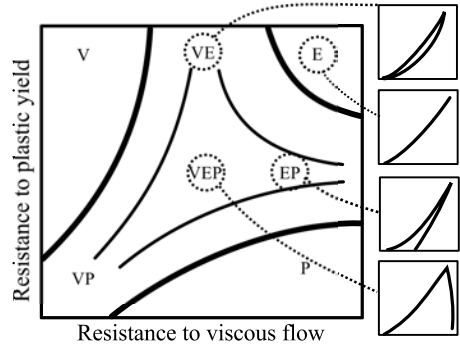


Figure 6.6. A behavior map detailing the viscous–elastic–plastic indentation space (Oyen and Cook 2009).

Such tests are becoming more important and relevant as technology continues to develop ways of interrogating materials and structures in ever finer detail. Jim Gordon reports the response of a Russian visitor to his lab on seeing the first electron micrograph of a dislocation in the structure of a crystal: “You are looking up the trousers of God!” Such intimate details, especially of biological materials that are so finely crafted from the molecular level up, become of greater interest as we develop better ways of looking at them. Hence, we can elaborate on ideas about their function and interrelationships and think about making our own versions. Nowhere is this process more true than with indentation, with which we can investigate and quantify stiffness, viscous properties, and fracture at submicrometer levels. Thus we are able to build up a picture of mechanical properties at different levels of structural hierarchy of “materials” such as bone and nacre and see how the mechanical interactions at various size levels combine to make such adaptable materials.

Now that we have the technology to measure the forces generated as a probe—round or pyramidal, pointed or blunt—is driven into a material, we can record the behavior over a wide range of material properties (figure 6.6) and derive information that we can integrate with data obtained from larger specimens (Oyen and Cook 2009), whose bulk properties are measured using methods outlined in chapter 1. We can measure continuum properties such as elasticity, viscosity, and plasticity as well as discontinuous properties such as fracture (figure 6.7).

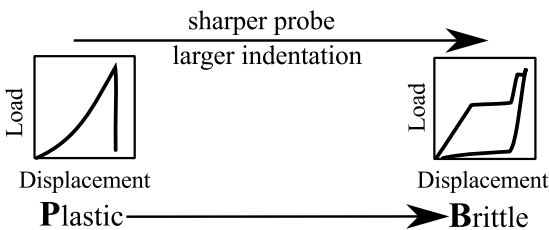


Figure 6.7 Map of the plastic-to-brittle transition in terms of the brittleness parameter (Oyen and Cook 2009).

6.2.3 WEIBULL ANALYSIS

Although many calcified tissues are very strong and stiff, they are still liable to break unpredictably at small strains. Because most fracture experiments involve introduction of a small crack into the test piece, and since the Griffith critical crack length of a brittle material may be only a few micrometers long (which is why the material is “brittle”), experimentation becomes difficult. Even though it would technically be possible to introduce such a small starter crack, it’s quite likely that there would be other cracks of similar size elsewhere on the specimen, and any of those undocumented cracks could start the Big One. We need a different approach.

In 1926 Pierce proposed the “weakest-link” theory to study the fracture of cotton fibers, which considered the fiber as a long chain made of several links. When stretched, the chain breaks at the weakest link, and the failure of this link leads to the failure of the whole chain. In 1951 Weibull described a statistical distribution that, together with this weakest-link theory, is widely used in failure analysis and reliability engineering. Weibull said that if n_σ represents the probability of failure of a chain of length L , $n_\sigma \cdot L_i$ is the probability that a link L_i units long will break. The chain will survive if no links break. This probability of survival is the same as the probability of survival, S , of each individual link. Because we can make the links as small as we want, when their length tends to zero, S tends to $\exp(-n_\sigma L)$, where L is the fiber length. Weibull proposed the following expression for n_σ :

$$n_\sigma = \frac{1}{L_0} \left(\frac{\sigma}{\sigma_0} \right)^m, \quad [\text{Eq. 6.5}]$$

where m is the Weibull modulus, σ_0 is the characteristic strength, L_0 is a reference length that accounts for the different lengths of fibers, and σ is the uniform uniaxial stress in the fiber. Because Probability of failure = 1 – Probability of surviving ($P_f = 1 - S$), the probability of failure of a fiber of length L_0 subjected to a uniform stress is

$$P_f = 1 - \exp \left[- \left(\frac{\sigma}{\sigma_0} \right)^m \right]. \quad [\text{Eq. 6.6}]$$

This equation is satisfactory for tension tests, but a three-point bending test is a nonhomogeneous uniaxial structure, so the imaginary links do not support the same stress. Therefore, the equation must be modified (Bazant and Planas 1998):

$$P_f = 1 - \exp \left[- \frac{1}{2(1+m)^2} \left(\frac{\sigma}{\sigma_0} \right)^m \right], \quad [\text{Eq. 6.7}]$$

where σ is the maximum stress at midspan (maximum stress in the beam). The distribution parameters can easily be obtained from a series of tests from which the breaking force can be calculated, and the corresponding maximum stress computed using the normal formula for three-point bending. The probability of failure can be calculated by sorting the stresses in ascending order and assigning ascending probabilities as $P_f = i/(n+1)$, where i is the position in the sorted list, and n is the total

number of values. By plotting $\ln(-\ln(1 - Pf))$ versus $\ln(\sigma)$, known as the Weibull plot, we then obtain a set of points that we can fit to a straight line:

$$\ln(-\ln(1 - P_f)) = m[\ln(\sigma) - \ln(\sigma_0)] - \ln[2(1 + m)^2]. \quad [\text{Eq. 6.8}]$$

The linear fit of the experimental values gives the Weibull distribution parameters, m and σ_0 . To obtain a reliable value for these parameters, a minimum of about 40 samples should be tested. This stricture is not commonly applied with biological materials, because specimens may be difficult to prepare. But there's no excuse for not using the transformation that gives a straight line, since the statistics of straightness are very powerful and easily assessed—much more so than some of the curves that are presented as the end product of a Weibull analysis. The higher the value of the Weibull modulus is, the more reliable and consistent is the material.

6.3 Mollusc Shells

Calcified tissues are found in most, if not all, of the invertebrate phyla in one form or another. Of these the most easily, and therefore the most extensively, investigated are the shells of molluscs. There are five main types of shell material (nacreous, prismatic, foliated, homogeneous, and crossed-lamellar; table 6.2 and figure 6.8) that show differences in structure and composition. These types of material can occur alongside one another in a single shell; in bivalves, for instance, there are three characteristic arrangements: (a) nacre inside, prismatic material outside; (b) foliated material with a little crossed lamellar material; and (c) crossed lamellar material, either on its own or with a little homogeneous material. Other combinations of material type are found within a single shell, but these three are the most common. The type and arrangement of material tends to be consistent with phylogeny: usually, all species within a family—and sometimes even those within a superfamily—have the same arrangement. Thin shells tend to be made of prismatic material with nacre or foliated material; very thick shells tend to be made of crossed lamellar material. Surprisingly, nacre is probably the most primitive of the shell materials and is composed of an intrinsically weaker ceramic. John Currey (1977) has studied the strength of nacre and crossed lamellar shell and has shown that the governing factor is the structure of the material. These two types will be examined in more detail.

To understand the way nacre works as a material, we need to be able to model it, which involves composite theory again. If we use stiffness values of 100 GPa for aragonite (Hearmon 1946) and 3 GPa for the material closest to the matrix—wet keratin matrix from fresh horn (Kitchener and Vincent 1987), the Voigt model gives us a Young's modulus of 95 GPa, and the Reuss model, 39 GPa. If we assume the matrix modulus of the dry nacre is that of dry keratin matrix (i.e., 6 GPa), the Voigt estimate is hardly affected; the Reuss model gives 56.5 GPa. Thus neither model predicts the actual modulus of nacre very well. However, both these models make the assumptions that the Poisson's ratio of both components is identical and that the fiber (ceramic) phase is composed of infinitely long particles. The shear modulus of the nacre matrix, which can be measured on a short beam ($S/D = 3$ or so), is 4.6 GPa (dry

TABLE 6.2
Types of mollusc shell material

<i>Type (figure 6.8)</i>	<i>Shape</i>	<i>Crystals</i>		<i>Protein matrix (% by weight)</i>	<i>Strength (MPa)</i>			<i>Stiffness (GPa)</i>	<i>Hardness (GPa)</i>
		<i>Calcite</i>	<i>Aragonite</i>		<i>Tens.</i>	<i>Comp.</i>	<i>Bend</i>		
Prismatic	Polygonal columns 100–200 μm by several mm long	*	*	15- μm sheet around each prism (1%–4%)	60	250	140	30	1.62
Nacreous	Sheets of tablets 0.3–0.5 μm thick		*	Thin layer between sheets (1%–4%)	130 (wet) 170 (dry)	380	220	60 (wet) 70 (dry)	1.68
Crossed lamellar	Plywoodlike lamellae 20–40 μm thick		*	Very tenuous (0.01%–4%)	40	250	100	60	2.5
Foliated	Long thin crystals in overlapping layers	*		Very tenuous (0.1%–0.3%)	30	150	100	40	1.1
Homogeneous	Fine-scale rubble (0.5–3 μm in diameter)		*	Very tenuous	30	250	80	60	0

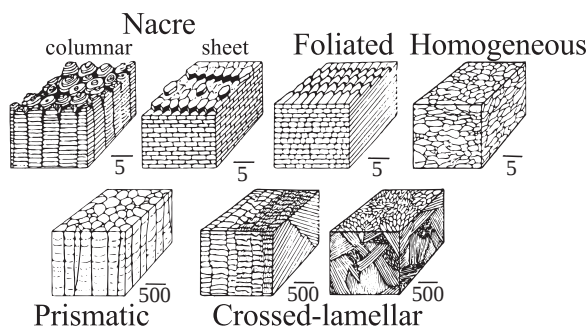


Figure 6.8. Structures of shell material in molluscs, from diagrams by Currey. Scale lines shown in μm .

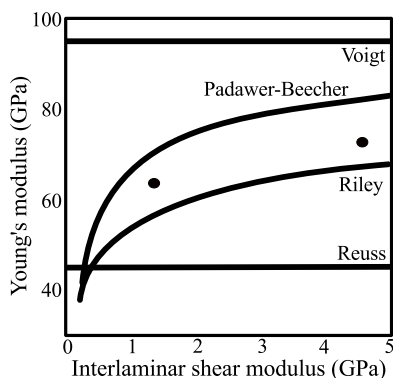


Figure 6.9. Prediction of the Young's modulus of nacre at different values of the interlaminar shear modulus. The dots represent experimental values with dry and wet matrix at high and low values, respectively, of the shear modulus (Jackson et al. 1988).

nacre) and 1.4 GPa (wet nacre) (Jackson 1986). Two relevant models (Padawer and Beecher 1970; Riley 1968) calculated over a range of matrix shear moduli yield the curves shown in figure 6.9 (Voigt and Reuss values are shown for comparison). The measured values for wet and dry nacre are also shown (Jackson, Vincent, and Turner 1988). Further modeling suggested that the strength of nacre can best be explained by the pulling out of the plates from between each other rather than the breaking of the bulk nacre straight across independent of the platy structure (Jackson, Vincent, and Turner 1988). This hypothesis was confirmed by models made with glass microscope slides stuck together with epoxy resin (Jackson, Vincent, and Turner 1989) (but see the later discussion).

Wet nacre loaded in tension is initially Hookean, then plastic up to strains of nearly 0.1 (and locally probably higher) (figure 6.10) In the plastic region the material appears milky owing to light-reflecting interfaces that develop inside the structure as the platelets are pulled apart (Currey 1977). The behavior of the material when it breaks depends on whether the crack travels across (the strong and tough direction) or between (the weak direction) the platelets. The work of fracture varies between 200 and 1250 J m^{-2} , depending on S/D ratio, degree of hydration, and orientation of the crack path (table 6.2). Several features combine to give this toughness.

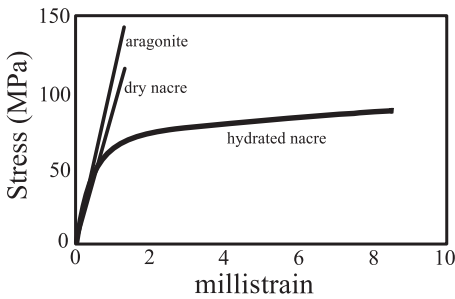


Figure 6.10. Stress–strain curve of nacre (wet or dry) in tension along the tablets compared with pure aragonite (Barthelat 2007).

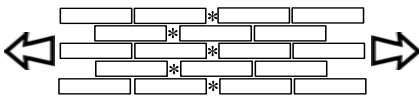


Figure 6.11. Failure of nacre if a crack can propagate through the material unhindered. This is what happens in three-point bending, in which the highest bending stress is in the center of the test piece.

1. The sheets are about $0.5\ \mu\text{m}$ thick, but the critical crack length of aragonite is about three times this value, so the sheets are not thick enough to contain dangerous flaws that could start a crack through the material (Currey 1977).
2. In three-point bending, the fracture is continually deflected to each side of its main path (Currey 1977). In addition, many plates are separated laterally (figure 6.11) (Jackson, Vincent, and Turner 1988).
3. A fracture that does not go through a sheet of ceramic goes through the matrix, which indicates that matrix and ceramic phases are well bonded (Currey 1977).
4. In fully hydrated nacre, when the matrix is fractured, it forms many ligaments that connect the two surfaces, which indicates that it is very compliant (Jackson, Vincent, and Turner 1988).

Currey's work was essentially not bettered for 30 years, although it was added to by Jackson, Vincent, and Turner (1988), and theoretical models were developed to account for the mechanical properties. In addition, the matrix was more completely characterized both chemically and mechanically, though the latter turns out not to be so significant as long as the matrix is hydrated. Recent work, however, performed with new physical and computational techniques, has refined our understanding and shown how pointless it can be to develop theoretical models in the absence of experimentation and observation.

The most important factor has proved to be the variable thickness of the aragonite plates (figure 6.12). As the nacre is deformed in tension, the plates slide and then lock against one another. The result is that the applied deformation is transferred to a different part of the specimen. Thus rather than creating the single crack that is observed in three-point bending, and which would be observed in a material with totally regular and orthogonal platelets (figure 6.11), the energy is absorbed and spreads damage throughout the material (figure 6.13), diminishing the likelihood of catastrophic failure (Barthelat et al. 2007).

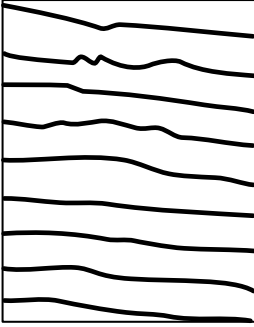


Figure 6.12. The platelets of nacre are not like bricks in a wall—the borders between them are, crucially, very uneven (Barthelat 2007).

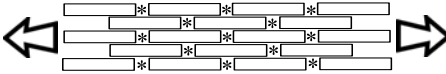


Figure 6.13. As nacre is deformed in tension along the platelets, they are pulled apart and lock against each other owing to the unevenness shown in figure 6.12. The deformation is transferred elsewhere, and the entire specimen responds to the strain (Barthelat 2007).

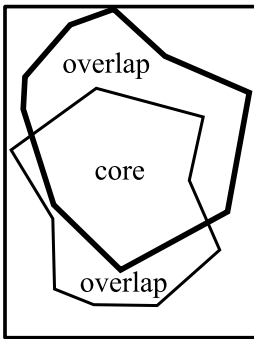


Figure 6.14. The irregular shape of nacre platelets ensures a statistical distribution of shear transfer between them (Barthelat 2007).

The matrix protein now assumes a different role. It isn't a glue that holds everything together so much as a lubricant that allows movement and may well enable self-repair (Kendall 1981, 1998). The formation of ligaments in the matrix probably helps distribute the strain more evenly through the structure. This locking mechanism of strain hardening relies on the overlapping of the platelets (by 30%–50%) from one layer to the next (figure 6.14), which gives rise to the “brick wall” appearance noted in sections of nacre. The overlap affects the mechanical response gradually because the plates are not all the same size and shape, so there is a statistical range of degrees of overlap. If the plates were all hexagonal and perfectly packed, the toughening mechanism would not be distributed but would kick in as a transition between totally elastic response and the plastic phase. A regular tiling could withstand strains that were about 50% larger but would absorb much less energy. The rate of energy

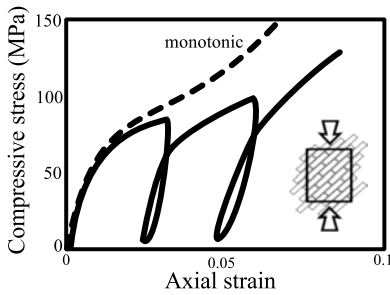


Figure 6.15. Compression stress–strain curves of nacre measuring interlamellar shear both monotonically and hysteretically (Wang 2001).

absorption in natural nacre can be seen even more clearly if the nacre is sheared cyclically (figure 6.15); the return leg of the cycle shows how much of the deformation is plastic, and represents energy absorption (Wang et al. 2001). Interestingly, the lumps and bumps (as opposed to profile waviness) observed on the surface of nacre platelets are probably not so relevant to energy absorption, since they tend to occur in the center of the platelets and do not contribute significantly to the locking mechanism, although they still strengthen the interface (Barthelat et al. 2007). But the question remains: How much can the model apply to columnar nacre, where there is no overlap between the platelets?

Thus we can add two more features to the previous list:

5. The platelets have a wavy surface, and so they lock against one another and allow strain energy to be spread throughout the bulk of the material.
6. The matrix is as much lubricant as glue; it may also transfer shear strain.

The fascinating and really quite frightening lesson here is that by making our vision of a natural material conform to our Cartesian worldview, we are still looking at biological systems and structures as if they were made by someone of poor intellect and imagination, whereas it is we who cannot appreciate the subtleties. This observation was made in the seminal *On Growth and Form* (Thompson 1959), where the asymmetrical (and therefore messy and “wrong”) arrangement of cells in an embryo or a meristem was shown to make excellent sense when surface energy is taken into account, using soap bubbles as the model—an early example of self-assembly! More recently, Friedrich Barth told a story about his research on the strain sensors of spiders, which comprise an apparently messy arrangement of subparallel slits of varying shape and straightness. This array deforms in a much more linear manner than the “cleaned-up” version of straight, parallel slits of constant width that his computer modeler insisted on working on! As always, the devil is in the detail, which we probably often miss when we “clean up” our observations to make the problems more easily soluble—but wrong! We have to be careful how much we create of the world that we are trying to describe.

When nacre is built into a shell it is laid down so that the sheets are parallel to the surface of the shell. Thus any crack that travels through the shell traverses the nacre across the platelets in the direction of greatest toughness. In the intact mollusc, catastrophic failure is very likely in whole, undamaged shells that behave like unnotched

beams at a large S/D ratio. Furthermore, control of crack propagation is made more difficult, since the muscles of a predator store significant amounts of strain energy and operate like a “soft” machine. You experience this process whenever you crack a nut between your teeth. Without sophisticated control mechanisms in your jaw muscles, your teeth would come together very hard when the nut shell finally breaks. Thus by the time a predator reaches the stress required to break a shell, it has large amounts of strain energy ready to feed into the advancing crack. When failure does occur, it is lethal to the mollusc, because outer shell materials, by all accounts, are not sufficiently strong or tough enough to arrest a fast-moving crack. Currey observed crack stopping in nacre, but only by reversing the normal direction of bending (Currey 1977). Because a whole shell normally breaks catastrophically, what is most important to the organism is not the detail of crack propagation, as Currey implied, but the amount of additional energy absorbed through ductility processes before fracture starts. Absorption of energy during the propagation of a crack may not be an option open to the organism, because once the failure load is reached, drastic measures are needed to arrest a crack that is being fed by large amounts of strain energy from the predator’s muscles. This may be the reason why in some species of mollusc the high work of fracture of nacre has been sacrificed for a strategy whereby cracks can be stopped before it is too late to do repairs. If pieces of broken shell become too widely separated (as they would after catastrophic failure), then it becomes impossible for the animal to lay down material from the inside to heal the gap. Molluscs containing crossed-lamellar material (see later discussion), however, have the advantage of a crisscross arrangement of laths within their shell that enables cracks to be stopped very effectively (Currey and Kohn 1976). The effect of the S/D ratio is also important in molluscs with long thin shells as opposed to those with short fat ones. If, for example, shells grow more in length than in thickness, then young shells with a small S/D ratio should be more ductile and able to absorb more energy per unit mass of shell than can old ones. Perhaps this is the reason why so many molluscs produce shells made only of nacre when they are young but add other types of shell material as they get older and larger.

The load-deformation curve in three-point bending of the crossed lamellar shell (figure 6.16) of the carnivorous gastropod *Conus* (Currey and Kohn 1976) is

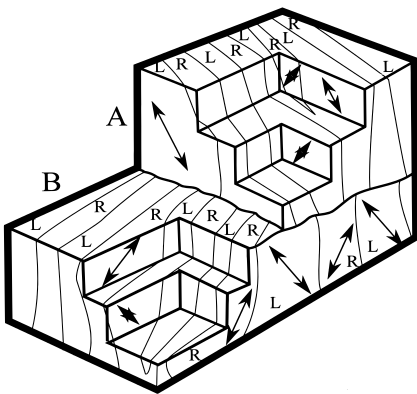


Figure 6.16. Hierarchical layering of crossed-lamellar shell. The main orientations of blocks A and B are orthogonal; in each block the lamellae are labeled L and R and are made up of laths whose orientation is indicated by the double-headed arrows (Currey and Kohn 1976).

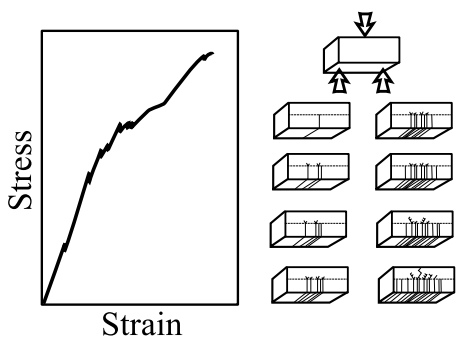


Figure 6.17. Bending fracture of crossed-lamellar shell (Currey and Kohn 1976).

saw-toothed initially, followed by a smooth region before failure. The sawtooth pattern has been found to be due to the changes in load as cracks run through the tensile layer between the lamellae and are then stopped by the middle layer; the following smooth region is correlated with the slight extension of one of these cracks into the middle layer; failure is due to the sudden propagation of this crack through the entire middle layer (figure 6.17). This interesting behavior is due to the morphology of this type of shell material. The basic morphology is somewhat like a plywood arrangement in which layers of shell, composed of laths nestled alongside one another (like the wood cells in a veneer), are arranged with the “grain” at 60° – 90° between them. A crack traveling between these layers will meet little resistance. But this layer gives way to another layer, in which the orientation of the plies is rotated so that the crack branches into either of the two directions that the “grain” dictates. The crack then has to break across the laths, effectively moving from one ply to the next, so its course is very tortuous. The crack then stops because there is insufficient energy to drive it farther, and a new crack starts, which meets the same fate.

This process is a typical “soft-sell” operation, in which the buyer is taken down an easy path from which there is no escape but doesn’t realize the ultimate expenditure until it is too late! For *Conus* this means that many cracks are started (a warning sign), most of which are stopped (giving time for escape) but develop individually into the “difficult” layer (absorbing strain energy) until ultimate failure. This approach to failure in biological systems is different from that in technical design. The idea of proliferating damage is counterintuitive, yet the outcome is to entrain a greater volume of energy-absorbing material and thus to avoid early catastrophe. This mechanism in *Conus* is similar to the processes in nacre (discussed previously), to the failure process in cross-grain impact of hardwood (section 5.5.2), and to the failure mechanism of antler (section 6.4.6).

It would be very interesting to compare prismatic material, with its high organic content, with the other types of mollusc shells, with their much lower organic content. In comparison with the sliding model of nacre, it may be that prismatic material relies even more for its toughness on the reorganization of the material during straining. Homogeneous material seems to have more in common with the rubble used by the original builders to fill the walls of old (Norman or Romanesque) churches—it is

extremely weak in tension, since it consists of small lumps of material rather like expanded polystyrene on a small scale and has very little organic material. Like rubble, it depends for its utility on its mass.

6.4 The Functional Design of Bone

6.4.1 WHERE'S THE MINERAL

Many books and articles have been written about bone. By far the best is by John Currey (2006). He deals with a number of structural and design aspects of bone that are not covered in a strictly materials approach—context is all.

The scaffold for bone is provided by type I collagen, within which crystals of hydroxyapatite are deposited. The three-dimensional arrangement of collagen fibrils in compact bone forms regularly ordered networks whose molecular assembly is highly analogous to that of nematic liquid crystals. Similar structures can be made *in vitro* (Giraud Guille et al. 2005), although, of course, it has been known for a long time that collagen in solution can form gel structures with varying degrees of overlap depending on salt concentration and pH (Wood and Keech 1960). The connection with liquid crystalline structures is crucial, since it gives clues about the conditions under which such relatively long range structures are formed. Even so, there is no direct evidence other than the shapes: both collagen and procollagen can spontaneously form liquid crystalline structures at high concentrations and in confined spaces. Charles Neville (1988) was probably one of the first to point out that a liquid crystal array with long-range order (which he called “monodomain”) could be generated only in confined conditions, such as when templated against a surface or confined within an extracellular compartment. A monodomain structure can be recognized by its behavior in polarized light: although it may display interference colors, these can be extinguished when viewed along the optical axis of the structure, with the polarographic analyzer rotated into the correct position. Such uniform extinction is impossible in a polydomain system. Neville gives a long list of monodomain structures in biological materials that explain the long-range order and layering.

The mineral phase in bone is commonly referred to as hydroxyapatite, though informed opinion (Lowenstam and Weiner 1989) is that most “hydroxyapatite” is in reality dahllite (carbonate apatite) or francolite (carbonate fluorapatite). Presumably, collagen has an array of charged groups, spaced in the same manner as the ions in the hydroxyapatite crystal lattice, that provides a nucleating site on which crystals can grow epitaxially (Glimcher 1984; Siperko and Landis 2001). Certainly, in bone the first crystals that form are precisely oriented with their crystallographic *c*-axis parallel to the long axis of the fiber, and this orientation seems to be retained as calcification proceeds, as shown by X-ray diffraction (White et al. 1979), neutron diffraction (G. E. Bacon, P. J. Bacon, and Griffiths 1979), and electron microscopy. These techniques show that the mineral crystals in mature bone are in the form of thin plates about 5.0 nm thick and about 35 (standard deviation about 15) nm along the *c*-axis (about the length of the gap region in the quarter-stagger model of the collagen fiber) suggest a model of the type shown in figure 6.18.

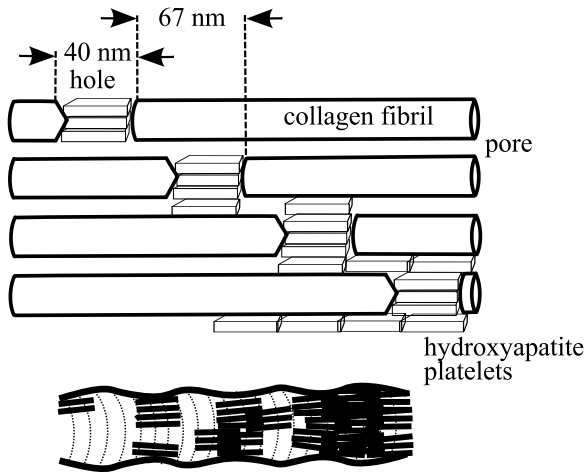


Figure 6.18. Hydroxyapatite crystals in bone (based on Glimcher 1984).

It is possible that only about 75% of the hydroxyapatite in bones such as that of a cow's leg is sited in these gaps (Katz and Li 1973), but the margin of error on this number is quite large. Nevertheless, the indication is that not all the bone mineral is intimately associated with the collagen. In addition, not all of it is crystalline; some is amorphous, as judged by X-ray diffraction. Bones of young vertebrates contain more amorphous material and become predominantly crystalline with maturity. As might be expected, this change in morphology is associated with changes in mechanical properties.

6.4.2 IS BONE A "SIMPLE" COMPOSITE?

The major part of mature bone is thus made up of crystals of hydroxyapatite embedded intimately in a collagen matrix, so it is possible to think of bone as a two-phase composite of crystals in a matrix. A complication is that bone has holes in it to carry essential services to the bone and marrow cells, so some correction has to be made for porosity. Even so, there has been much agonizing over which phase might be the principal load bearer. A mind-bogglingly simple experiment is to boil the bone (or, expressed professionally, to autoclave it—which means to boil it at 110°C —which doesn't make much difference, since soluble collagen melts at around 70°C), whereupon you find that the bone may (or may not) still be intact. Presumably, if it's intact and strong, then the crystals are sufficiently well packed to carry loads. The simple composite theory outlined in chapter 5 and the values given in table 6.3 suggest that the minimum volume fraction of hydroxyapatite necessary for it to act as a true reinforcement is about 0.35 (Wainwright et al. 1976), which is almost exactly the volume fraction calculated (Katz and Li 1973) to be formed within the collagen fibrils found in antler bone—so go boil an antler! According to Katz, it should just fall apart. I don't

TABLE 6.3
Material properties of the major components of bone

Strength of hydroxyapatite	0.1 GPa
Stiffness of hydroxyapatite	130 GPa
Ultimate strain of hydroxyapatite	10^{-3}
Stress in collagen at strain = 10^{-3}	1 MPa
Strength of collagen	30–50 MPa

know of anyone who has done this, and it would be best to use fresh antler so that the collagen isn't hydrogen-bonded owing to dehydration. As for the cow leg, since its volume fraction of hydroxyapatite is nearer 0.5, the mineral phase is probably the main load bearer. But Eq. 5.3 has a number of assumptions built into it, mainly that the crystals are long enough to act as fibrous reinforcers (i.e., that there is effective transfer of stress from collagen to crystal to collagen). It is possible that the hydroxyapatite crystals are long enough, but they are probably not surrounded by the collagen in the same way that fibers in a composite material are surrounded by amorphous matrix. However, there seems to be no argument against simply considering bone as a composite filled with particles of no particular shape, whereupon it conforms to a phenomenological model (Braem et al. 1987) and the pragmatically similar, though philosophically distinct, Hashin-Shtrickman model (Katz 1988). Both models are shown in figure 6.19. In a consideration of bones covering a wide range of mineralization, the general trend of variation of modulus versus mineral content follows the latter two models (figure 6.20) (Currey 1979, 1984a), although conversion of ash content to V_f of ceramic is a task fraught with difficulty, since the proportion of voids in bone can be very variable. Some estimates are shown in figure 6.21.

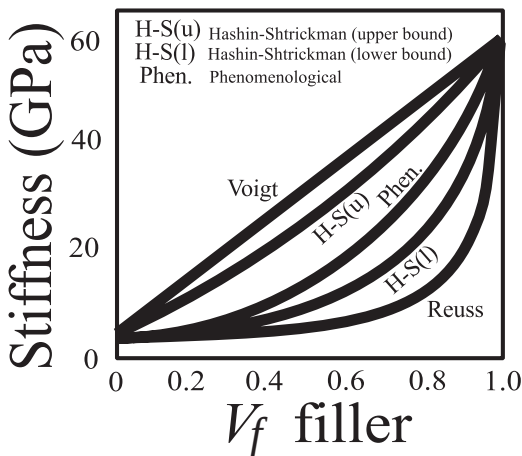


Figure 6.19. Stiffness of a ceramic composite based on a variety of models, none of which quite works (phenomenological).

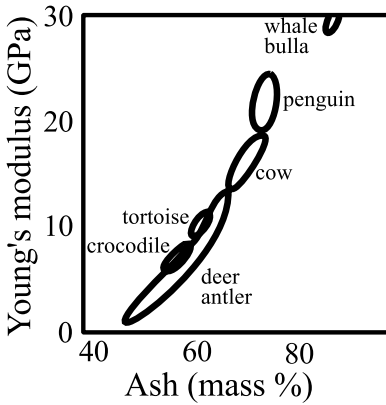


Figure 6.20. Stiffness of a variety of bones with varying ceramic content (Currey 1984).

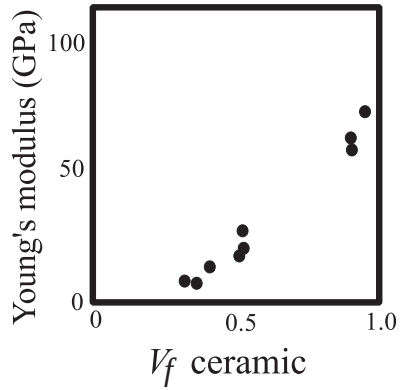


Figure 6.21. Volume fraction of mineral versus stiffness in bone, nacre, tooth, and aragonite (Currey 1984).

The main reasoning being followed by most workers in understanding the mechanical design of bone is that it is basically some sort of fibrous composite, yet the mineral fraction of bone is predominantly platy, like nacre, only much smaller. Only the collagen gives a fibrous texture, and the collagen is only about 1% as stiff as the mineral. Judging by the fracture surface of antler, the fiber analogy can hold only if we view the fiber itself as a composite reinforced with platelets. Exceptions may exist in some of the bones with a high V_f of mineral, such as dentin. Analytically, it seems, we are still confused, albeit on a somewhat higher plane.

6.4.3 MORPHOLOGY OF CORTICAL BONE

All the correlations between bone parameters discussed so far have neglected any structure of the bone at any level of organization higher than the mineral-impregnated collagen fiber. In fact, bone has a complex hierarchical structure, which obviously affects its mechanical performance: the density and arrangement of packing of the mineralized fibers control the direction and magnitude of the stress that can be sustained and the way it is transmitted through the bone. Neutron diffraction studies of human scapula show that the c-axes of the hydroxyapatite crystals lie preferentially along the directions of pull of the attached muscles (G. Bacon, P. Bacon, and Griffiths 1979; Bacon and Griffiths 1985). In areas where two muscles act in different directions the crystals are found to be oriented in two groups coordinated with the directions of pull (figure 6.22). Although there is some disagreement as to the morphology of bone, the following model is generally accepted (figure 6.23). The fibrils are arranged in two main ways: (a) in layers with a preferred orientation, with the layers aligned with one another and varying in direction much like the layers in insect cuticle or the secondary cell wall of wood cells (lamellar bone), and (b) more

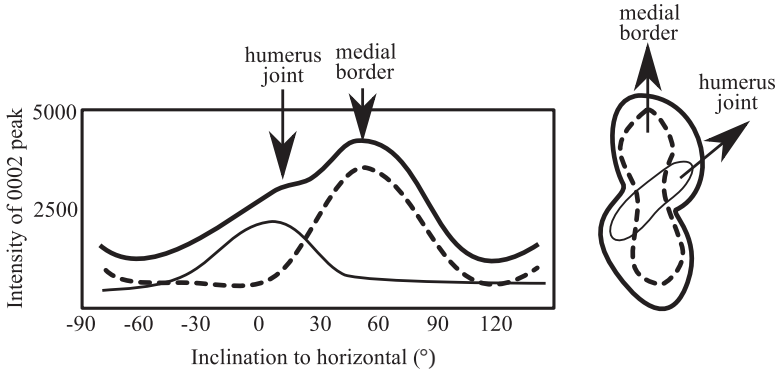


Figure 6.22. Distribution of orientations of the c-axis of mineral crystals in bone taken from the human scapula (Bacon et al. 1979).

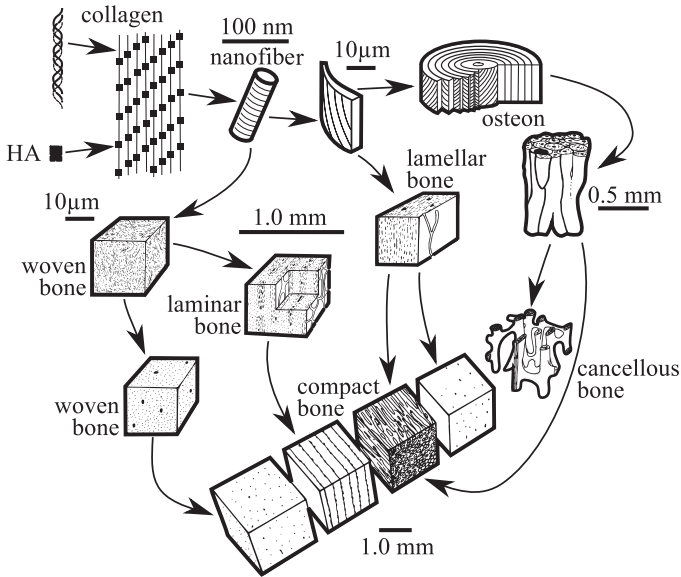


Figure 6.23. Hierarchy and complexity in the structure of bone.

or less random (woven bone). Lamellar bone can be built into sheets in one plane (primary lamellar bone) or into cylinders like wood cells (Haversian bone) or mixed with woven bone (laminar bone). All types of bone structure are found in compact bone; the more open-textured bone found in the center and ends of long bones and in vertebrae is constructed only of lamellar bone (primary lamellar) and is known as *cancellous* bone.

Haversian bone is probably the most common form of bone in the human skeleton and is widely found in other vertebrates. It is usually a replacement bone in that it develops after the bone has largely been calcified. Hence the cylindrical units that make up Haversian bone—the osteons—are, strictly speaking, secondary osteons. Down the middle of the osteon runs a blood vessel. The bone is laid down and maintained by osteocytes that are situated among the concentric layers of fibers and send out processes into the surrounding bone. The orientation conforms to the “helicoidal” or twisted nematic model of Bouligand (Giraud-Guille et al. 2005, 2008). Nevertheless, the complex of annular layers, cells, and blood vessels is called the *Haversian system*. Adjacent systems are delimited by the cement line, a sheath of calcified mucopolysaccharide that apparently lacks collagen. The osteon so formed can be branched, but it is usually oriented along the long axis of the bone.

6.4.4 MORPHOLOGY AND MECHANICAL PROPERTIES

It’s sad but true that the problem of accounting for the mechanical properties of bone, accepted as a high-performance material, has received scant attention since the late 1980s. At a global level the amount of hydroxyapatite is the controlling factor in the stiffness of bone. Indeed, one might expect morphology to be secondary to mineral content in dictating stiffness. Currey (2006) reported stiffness, ultimate stress, and the strain at yield in cow bones composed of differing amounts of laminar bone and Haversian bone. He found that the effects on the mechanical properties of reconstruction and the falling ash content that accompanies it are inseparable. In other words, the morphology of the bone seems to have little effect. By far the most important is the fact that laminar bone yields about 67% ash, and Haversian bone, 93%.

This independence from morphology is not so apparent at smaller scales, however. The variation in the Young’s modulus of Haversian bone at various orientations to the longitudinal axis of the bone (figure 6.24, solid line), found using an ultrasonic technique (the speed of sound through a material is a direct function of its stiffness), shows that the stiffness does not fall off as quickly with change in orientation from the long axis as might be expected from fiber composite theory (dashed line) (Bonfield and Gryn timer 1977). But the composite model used had a single fiber orientation,

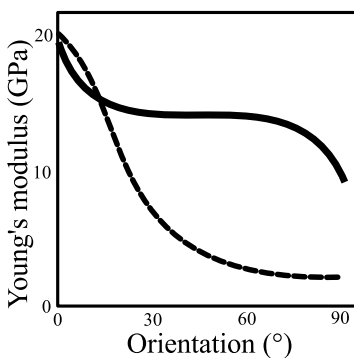


Figure 6.24. Comparison of stiffness with orientation relative to the long axis of a unidirectional fibrous composite (dashed line) and osteate bone (Bonfield and Gryn timer 1977).

whereas the osteons of compact bone have more complex and varying fiber orientations. This experiment therefore confirms what has already become clear—that a simple-minded fiber composite model is inadequate for Haversian bone.

6.4.5 VISCOELASTICITY

Bone is certainly viscoelastic. Cortical (Haversian) bone from the cow creeps at the cement line (the junction of osteons) under load (Lakes and Saha 1979) and has a viscoelastic spectrum whose peaks (such as they are) can be ascribed to individual morphological elements according to size—for instance, lamellae and osteons (Lakes and Katz 1979)—which suggests that these elements have some mechanical independence. However, viscoelasticity is more likely to be relevant to fracture than to standing still for a long time: How do fracture properties vary with strain rate? This variation is not the same as the accumulation of damage over a period of time without allowing time for the damage to heal, it's about fracture that progresses faster than the toughening mechanisms of bone can react. It turns out that the plasticizing effect of water, which facilitates the viscoelasticity, is crucial for control of fracture.

6.4.6 BROKEN BONES

Currey's attention was first drawn to the toughness of antlers (which are not horns, because they are made of bone, are shed and regrown every year, grow from the tips of the tines rather than the base, and are branched) by the uses to which humans have put them—notably, as pickaxes and a material from which to make long-lasting combs. Both these uses demand a tough material even though, as an item of display, antlers need be made of nothing more sophisticated than, as Currey says, waterproof cardboard. But when stags fight, the clash of antlers reverberates for miles around. Clearly, the antlers absorb much impact energy and transfer it to the muscles of the stag's neck, where most of the energy of fighting is absorbed. Thus the antlers must be tough if their owner is to win the confrontation. Impact fracture tests (figure 6.25a) show that antler breaks into only two pieces, which suggests control of the crack path, whereas cow leg bone shatters in an almost glassy manner (figure 6.25b) (Watkins 1987). Antler is the least mineralized and thus the least stiff of mammalian bones, which implies that it can reach a relatively high strain. The mineral phase seems to be confined within the collagen fibrils; the fracture surface (figure 6.25c) shows fibers less than 200 nm in diameter, which is only slightly larger than the size of fiber reported for mineralizing turkey tendon (Weiner and Traub 1986), and the amount of mineral corresponds to the space available within the fiber (Katz and Li 1973). It seems that at this low level of mineralization there is no cementing mineral around the fibers, so they remain free and unadhered. This lack of cementation is also indicated by the tensile stress-strain curve of antler—it shows significant plastic yielding, presumably because the fibers stretch and pull out (Watkins, 1987). More recent work using time-resolved synchrotron small-angle X-ray diffraction on bone under tension has confirmed this model (Krauss et al. 2009), which shows that global strain (after yield) can be 50% greater than local strain of individual fibrils, which

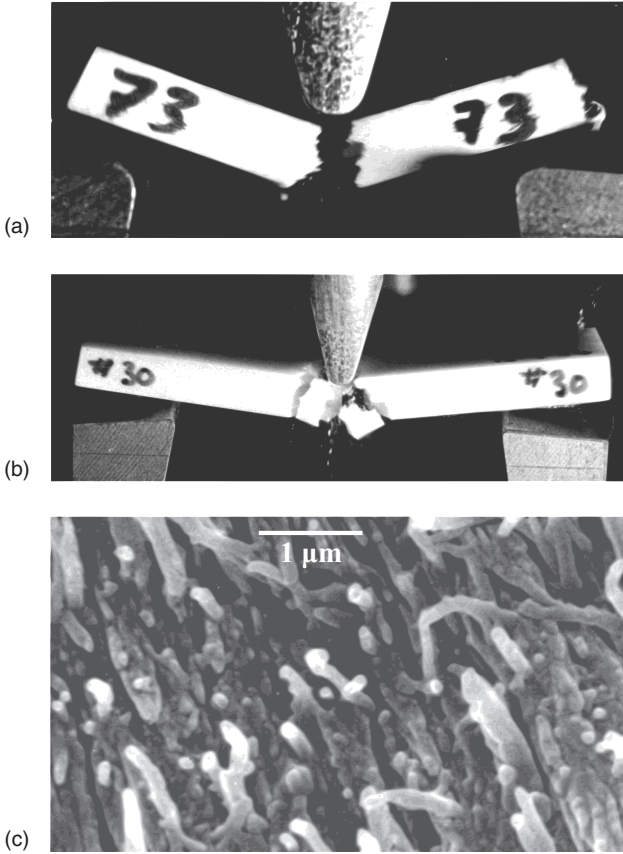


Figure 6.25. (a) Impact fracture of antler bone; (b) Impact fracture of cow leg (osteate) bone; (c) fracture surface of antler. All samples were wet (Watkins 1987).

appear not to yield (figure 6.26). The macro-yield occurs throughout the specimen, which is, of course, impossible without some additional factor. Global yielding of this sort implies reduction in the overlap of adjacent fibrils, which would result in local weakening and thus premature fracture at a single site. The bone doesn't fracture, so local yielding must get locked at the 50% level. Thus the strain energy has to be dissipated elsewhere in the system, rather like the mechanism shown to occur in nacre (Barthelat et al. 2007), and fibrils are pulled apart end from end through the bulk of the bone. John Currey noted that both bone and nacre become opaque and white (reflecting light) as they yield and deduced that the internal components separate, leaving holes. Barthelat showed this to be very probable for nacre, and Thurner showed this to be true for bone (Thurner et al. 2007) Because the collagen fibrils contain hydroxyapatite only in the gap regions, it is difficult to envisage the mechanism by which this might occur, other than to say that Poisson's ratio has to be involved somewhere, rather as in the model for fibrin (section 4.3), which showed

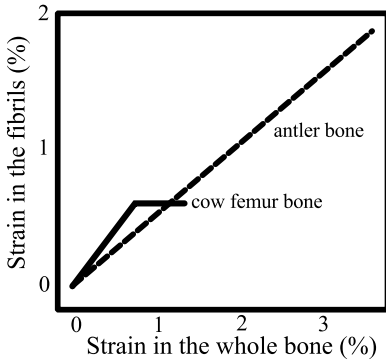


Figure 6.26. X-ray diffraction of bone in tension has shown that strain in the whole bone can be 50% greater than strain in the individual fibers (Kraus et al. 2009).

that it is quite possible to have different Poisson's ratios at different levels within a hierarchical structure.

However, the low level of mineralization also means that antler bone is not so strong as bones with higher mineral (= ash) content (Currey 1984). Cow leg bone has an intermediate stiffness and degree of mineralization (figure 6.20). Although it is not so tough as antler, it is stronger and can withstand higher forces: it does not deflect so much under load. Indeed, it does not need to be as tough as antler, since in life it is covered in flesh and skin that cushions shocks to a great extent. The ear bone or tympanic bulla of the whale is never submitted to such shocks. Its function requires that it have high inertia (John Currey paints the evocative picture of this massive piece of bone staying still in the water while the whale vibrates around it!), so it is very massive and has more mineral in it per unit volume than any other bone. But although it is very stiff (figure 6.20), it becomes weak and brittle and has a smooth fracture surface not unlike a glass.

It is symptomatic of the sort of compromise that biological materials have to achieve that cracks probably commonly start from the holes and tubes that bring the bone its lifeblood. If bone is broken slowly, it is possible to watch cracks starting and progressing through the bone. In one study, cracks started to form at about 60% of the failure load, and in another instance a crack 230 μm long was seen moving from a small canal about 25 μm in diameter. In most cases the cracks started from the cavities occupied by the osteocytes and moved between the lamellae of the osteons. This latter mode seems to be more common in fracture at low strain rates, and the cracks are then deflected around the osteons between the lamellae. More commonly, cracks can be seen to originate at blood vessels, which are anywhere from 20 to 100 μm in diameter. This conclusion was reached independently by Bonfield (Bonfield and Datta 1976) using an analysis based on Griffith's ideas of fracture (section 1.6) rather than direct observation. He calculated an "intrinsic crack length" from which further cracks could start and found it to be about 340 μm , which is nearer the size of the blood vessels than of the osteocytes (3–5 μm). This finding was confirmed with a more direct and elegant technique—double notching in a four-point bending beam (Nalla et al. 2005). The middle section of a beam in four-point bending experiences

uniform stress, so that two notches side-by-side experience exactly similar loadings. However, one of them will cause a fracture before the other, so the remaining notch will retain the undeveloped damage of a notch just before failure.

That bone is viscoelastic suggests that the way it breaks, and its toughness, should be a function of strain rate. It should also be dependent on the degree of viscoelasticity (i.e., the amount of plasticizer (= water) in the collagenous matrix) in the same way that nacre is (table 6.4) (Watkins 1987). At high strain rates, achieved by impact loading of various sorts (including the use of explosives), cracking does not follow any particular path through cow leg bone; at low strain rates it is very convoluted and shows a very rough surface. Osteons are pulled out whole very much like fibers pull out in artificial composites. Fragments of osteons can also be seen, which indicates that the crack did not propagate perpendicularly through all the bone constituents. The fracture surface of cow leg bone produced by failure at high strain rates shows no evidence of pullout, and the surface is much smoother—like that of the tympanic bulla of the whale. Evidently, these differences are a result of the viscoelastic response of the bone to different strain rates. In a study on cow leg bone Robertson and Smith (1978) found that at high strain rates the stress-strain curve was linear with a brittle failure typical of a glass; at low strain rates, with ample time given for plastic relaxation to occur, the stress-strain curve was not linear—the bone yielded and supported load to higher strains. The transition between these two modes of fracture occurs at a strain rate of around $2.5 \times 10^3 \text{ s}^{-1}$ (i.e., it would take 4 s to reach a strain of 0.01).

It seems reasonable to think that this strain-rate dependence will be reflected in the work required to fracture the bone, but opinion seems to be divided. It appears that the work of fracture is mostly related to strain rate, but it seems likely that, as Currey has pointed out, the relationship between strain rate and various other mechanical parameters depends strongly on the structure of the bone—whether it is osteate, lamellate, or intermediate in some way. These structures can produce very different effects. It is also important that the sample have time to channel all its stored strain energy into the processes of fracture; otherwise the energy may be overestimated. Behiri and

TABLE 6.4
Comparison of two types of bone and whale bulla

<i>Specimen</i>	<i>Porosity (%)</i>	<i>Stiffness (GPa)</i>	<i>V_f Mineral</i>	<i>Toughness (kJ m⁻²)</i>	<i>Bend strength (MPa)</i>
Fetal calf (a)	21.3	5.9	0.35	—	—
Antler (b)	—	7.4	—	6.9	179.4
Antler (wet) (c)	—	7.7	0.4	13	240
Antler (dry) (c)	—	12.8	—	2.14	—
Adult cow (a)	19	20.5	0.51	—	—
Adult cow (b)	—	13.5	—	1.71	246.7
Whale bulla (a)	—	30	0.91	—	—
Whale bulla (b)	—	31.3	—	0.2	33

Bonfield (1982) reported that the toughness of cow leg bone increases from 0.63 to 2.88 kJ m^{-2} as the velocity at which the crack tip travels also increases, up to a value of about 1.2 mm s^{-1} . At this velocity the propagation of the crack becomes unstable, the fracture surface changes from being rough to glassy, and the fracture toughness drops to about 0.2 kJ m^{-2} . On balance, it seems that osteate bone is weaker at higher strain rates, but, again, such factors as the size of the osteons can affect the result—larger osteons seem more prone to brittle fracture, presumably because the path of the crack is not deflected so often.

The final consideration in considering fracture is the implied role played by the hierarchy. The interfaces between individual collagen fibers with their burden of hydroxyapatite can be bridged by the addition of mineral, and the interfaces between the laminae of the osteon can also be bridged, as can the interface in secondary bone between the osteons at the cement line. Each of these interfaces (and there are more between different types of bone, as well as mixtures of interfacial bridging in any one piece of bone) can facilitate or stop a crack, depending on orientation and speed. Bone is not uniform—its morphology and mechanical properties can change over very short distances. It's not surprising, therefore, that the reported values and behaviors are variable. One trend noted by Koester, Ager, and Ritchie (2008) is that the critical stress intensity of bone has increased with time—more recent results return higher values. Unfortunately, the authors don't report the size of the samples used over the 35-year period considered, but it's reasonable to think that modern technologies allow more accurate measurements to be made on smaller samples. Size matters.

6.5 Teeth

In the other major ceramic materials of vertebrates—tooth enamel and dentin—tensile forces of any appreciable magnitude occur much less frequently. The major requirements of a tooth are that it should have a hard surface for cutting and grinding (whatever they might be in physical terms) and that it should be durable (tough) enough to last the lifetime of the animal. The human tooth (figure 6.27) is a fairly basic type with dentin core (Young's modulus of about 20 GPa) and enamel capping

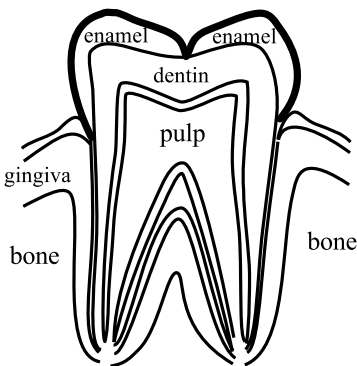


Figure 6.27. A standard mammalian tooth in section.

(Young's modulus of about 95 GPa). Dentin and bone are similar in overall composition, but enamel contains much more mineral. Together the dentin and enamel of the tooth are subjected to loads of 20 MPa, 3000 times a day (fewer if you are on a diet). Even so, fracture of sound teeth is very rare, partly owing to the hardness and stiffness of enamel and partly to the toughness and relative compliance of dentin.

Dentin is more uniformly constructed than bone, but the crystals (of dahllite, a carbonated apatite) are probably much thinner—about $2 \times 50 \times 25$ nm—in a matrix of collagen-forming fibrils 50–100 nm in diameter. The dentin is permeated by tubules 1–2 μm in diameter that run from the dentin–enamel junction to the pulp. The tubules are surrounded by a highly calcified zone (the peritubular dentin) about 1 μm thick in a matrix of randomly oriented crystals in mucopolysaccharide and collagen; the latter is oriented in planes parallel to the surface of the dentin. The tubules contain liquid. The main form of toughening seems to be crack bridging, by ligaments if the crack runs at an angle to the collagen fibrils (figure 6.28).

Enamel has an even more complex structure (figure 6.29). The spaghetti-like crystals of dahllite are at least 100 μm long but may be only 50 nm in diameter (Daculsi et al. 1984), so that each crystal extends from the surface of the tooth to the junction with the dentin. Such a high aspect ratio allows these crystals to assume a variety of curvatures along their length, which may account for previous reports of a variety of orientations of smaller crystals within the large components into which these crystals are organized. These components are various sorts of rods and sheets, depending

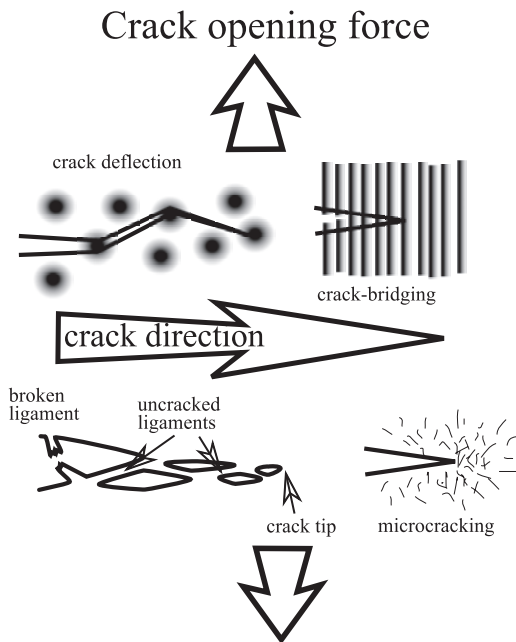


Figure 6.28. Failure mechanisms observed in tooth. The tensile force is represented as vertical; the cracks therefore run horizontally.

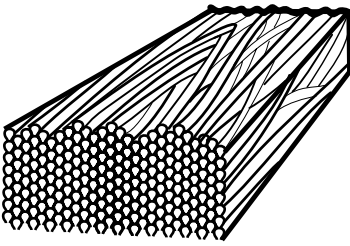


Figure 6.29. Structure of enamel.

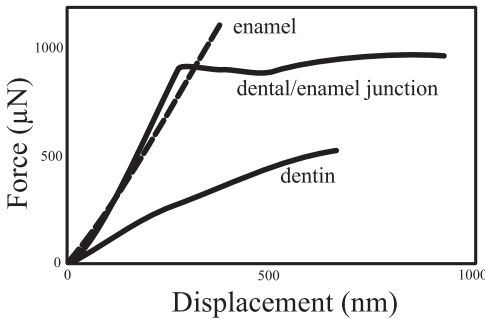


Figure 6.30. Force-displacement curves of enamel and dentin, in an effort to understand the mechanical nature of the interface between the two (Chan 2010).

on the animal. The crystals can branch and fuse, leading to the formation of pyramidal shapes whose wide base projects toward the dentin layer. In mammals the arrangement of these rods and sheets can be complex and various; in some instances the array of rods is completely three-dimensional. The interface between the dentin and the enamel presents some difficulties, since the stiffnesses are so different. One would expect problems with this sort of compliance mismatch, especially because the interface seems fairly abrupt (Chan, Ngan, and King 2010). The collagen fibrils from the dentin extend across the interface and stop where the enamel crystals start. Force-displacement plots of microcantilevers (figure 6.30) show that the junction yields significantly, probably owing to the collagen, and that this provides the compliance matching.

6.6 Eggshell

If the tooth is difficult to analyze and test, the eggshell of birds must be nearly impossible. Moreover, since materials scientists have by and large kept clear of mechanical tests on eggshells, thus demonstrating a clear understanding of the difficulties of such tests, the literature on the strength and other properties of the shell is dominated by half-boiled notions, mostly from biologists and agriculturalists. However, there are rational ways around the problems if experimenters keep to their areas of skill and expertise.

The eggshell is made of calcite, about 96%–98% by volume, and the rest is hydrated organic material, but the morphology of the shell material (figure 6.31) is

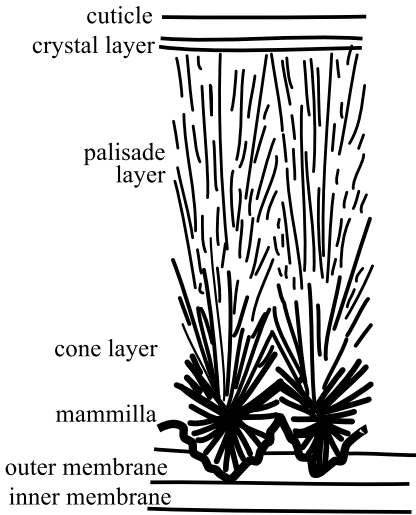


Figure 6.31. Section of a hen's egg showing the orientation of the crystallite texture.

complex and mechanically enigmatic. The organic material is spread throughout the shell, so that if the carbonate is dissolved, a “ghost” of organic material remains. The fracture surface of the mineral varies throughout the thickness of the shell: in the cone layer the surface is like that of prismatic mollusc shell; in the main thickness of the shell the fracture surface appears much less ordered, although the orientation of the calcite crystals is, in fact, very highly ordered (Silyn-Roberts and Sharp 1985a, 1985b). It seems possible that the fracture planes are defined by the distribution of organic material. The tips of the mammillar cones are connected by a highly fibrous membrane. The eggshell as a whole has a complex shape and is brittle, so it is very difficult to prepare a sample for mechanical testing. Any strip or beam cut from the shell will have a highly complex shape and may well contain microcracks as a result of preparing the specimen. Most shells are too thin to allow a rectangular cross section to be machined; in addition, it is difficult to decide which part of the shell section actually takes stress.

These factors combine to make standard materials tests impossible. Nanoindentation is, of course, not dependent on shape, and produces an elastic–plastic indentation curve (figure 6.32) that yields a modulus of about 50 GPa over the entire shell and through its thickness (Severa et al. 2010) with a standard deviation of about 12%. Tests on whole eggs gave a modulus of about 55 GPa but with a standard deviation of about 40% (Macleod, Bain, and Hancock 2006). These measurements required several methods and a variety of orientations but resulted in no differences in stiffness. The answer therefore seems to be that the complex crystalline structure of the eggshell doesn't affect stiffness, which leads to the deduction that it's the amount of ceramic that matters.

From the point of view of the egg, its intention is to stop anything from getting in, which is of practical importance for commercial egg handlers. From the point of

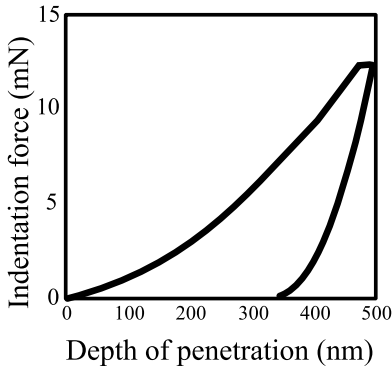


Figure 6.32. Nanoindentation of a hen's egg (Severa et al 2010).

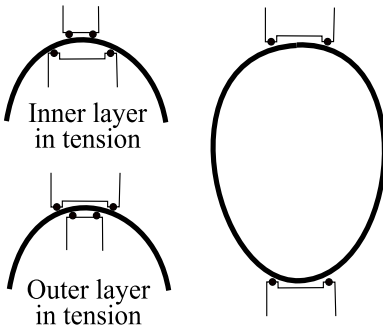


Figure 6.33. Loading geometry of eggshell used by Entwistle et al. (1995).

view of the chick, its intention is to get out at all cost, which is of practical importance for breeders. Measurements of the strength of eggshell therefore fall into two groups. And, at last, someone used Weibull analysis properly to analyze fracture properties of a brittle material!

Loading the eggshell using a mounted concentric pair of Teflon rings, one outside the egg and the other inside (giving a sort of circular four-point bending geometry, figure 6.33), Entwistle measured the fracture stress of the outer surface as 27.3 MPa and of the inner surface as 30.4 MPa (Entwistle, Silyn-Roberts, and Abuodha 1995)—not much difference. A similar method of loading a whole egg gave the strength of the inner layer as 36.7 MPa, so it looks as if the chick will have a difficult job. In a truly excellent study, Niall Macleod analyzed the problems from the industrial point of view—What damage is important? When does it occur? How does it relate to the eggshell? What can be done to ameliorate it (Macleod, Bain, and Hancock 2006)? Major cracks start from microcracks that can be initiated by a much smaller load than that required for the shell's failure. The microcracks initiated by a point contact extend over a diameter of 1.5 mm. Any probe smaller than this diameter can puncture the shell without causing catastrophic cracking; a probe of larger diameter acts like an infinitely large platen and causes catastrophic cracking. Therefore, a subliminal form of cracking is probably initiated by normal handling and grading of eggs, since

the Weibull modulus (an estimate of the spread of the fracture data) is much smaller for eggs after grading than before. A more theoretical analysis shows that these microcracks probably start from the intermamillary crevices. If these crevices could be closed up by increased deposition of mineral early in the development of the egg, a process that seems to be under genetic control, then the microcracking could be restricted. This is important because, among cracks, micro- can lead to macro-, and microcracks can allow bacteria to enter the eggs.

As for the chick, before attempting to hatch, it very wisely erodes away about half the thickness of the shell and incorporates the calcium within its own tissues, so the preceding values are not necessarily relevant to hatching. The chick escapes from the egg by chipping away a circle at one end of the egg and pushing on the disc formed so that the shell tears along the dotted line, which can be simulated in a mechanical test (figure 6.34). Quail and pigeon eggs are made of much tougher material than those of hen or duck (figure 6.35), and this difference is reflected in the hatching behavior of the chick. Hen and duck chicks need peck only partway around the shell before pushing off the disc to emerge; quail and pigeon chicks have to peck all round the shell before pushing. Thus, as any gourmet will tell you, crack propagation is easier in duck and hen eggs than it is in quail or pigeon eggs. The reason for this is obscure,

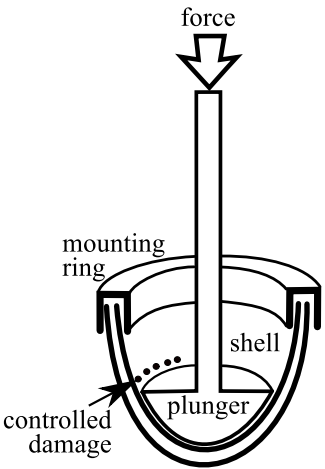


Figure 6.34. Loading geometry of eggshell used by Bond et al. (1986).

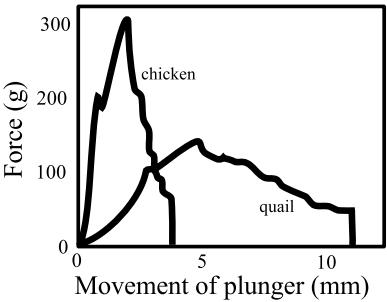


Figure 6.35. Fracture of eggshell using the geometry of figure 6.34 (Bond et al. 1986).

though it is most likely due to small differences in the amount and composition of the organic material. There seems to be no difference in the appearance of the broken surfaces of the two classes of eggshell (Bond, Scott, and Board 1986).

6.7 Echinoderms

Some of the most intriguing ceramics are produced by the echinoderms. Their skeletons are composed mainly of magnesium-bearing calcite and contain less than 1% of organic material. Individual components (plates from the test, spines, teeth, larval spicules, etc.) behave as single crystals when examined by X-ray diffraction or in polarized light. But when they fracture they behave in a number of ways. Wet pieces of *Echinaster spinulosus* test were loaded and fractured. If the pieces were fractured immediately, the fracture surface was smooth and conchoidal, typical of a brittle (though amorphous or glassy) material. But if the pieces were allowed to stress-relax before they were fractured, the fracture surface was rough, with many small needles that stuck up about $0.36\ \mu\text{m}$ above the surface, arranged as if they represented the broken ends of concentric lamellae (O'Neill 1981). The inference is that the needles are surrounded by a viscoelastic material that deforms during the stress relaxation, producing weak interfaces. This deduction is supported by indentation before and after extraction of the protein by NaOCl. Indentation causes longer cracks in the extracted material, as quantified by the critical stress intensity: for the intact material it is $0.97 \pm 0.04\ \text{MPa m}^{0.5}$, which drops to $0.68 \pm 0.05\ \text{MPa m}^{0.5}$ after extraction (Wang 1998). Thus the calcite must be polycrystalline with protein between the crystals. The puzzle is, How do the crystals (if they exist as separate entities) grow in such perfect register?

One way to investigate the mechanism is to grow the crystals yourself. Berman, Addadi, and Weiner (1988) grew calcite crystals in vitro in the presence of matrix proteins both from *Mytilus* (mussel) shell and a sea urchin shell. The calcite grown with urchin matrix protein was tougher and broke in a glassy manner rather than cleaving like a crystal. The *Mytilus* protein also affected the fracture behavior, but the resulting crystals crumbled instead of cleaving. The inference was that echinoderms make a "molecular composite": they lay down protein on the growing surface of the crystal, which is continually overgrown by more calcite. Perhaps discontinuities are generated at the growing surfaces, but they are not big enough to upset the orientation of the crystals significantly (Berman et al. 1990). Thus echinoderms manage to have the strength and stiffness of a ceramic, but without the brittleness.

Sea urchin larvae form an endoskeleton composed of a pair of spicules. A Young's modulus of 36.3 GPa was estimated for these calcitic spicules from E/II , where I (the second moment of area) was calculated independently from measurements made by electron microscopy. This value is about half to a third of the modulus of pure calcite, which leads to successful modeling of stiffness by the Reuss model (Emlet 1982). The flexural stiffness of fenestrated spicules (i.e., spicules with holes in them) is approximately three times greater than that of simple spicules. This increased flexural stiffness is due to structural, not material, differences between the spicules. At the

material level, this calcitic tissue behaves as a composite with reduced stiffness but increased strength compared with inorganic calcite. At the structural level, the porous nature of the tissue increases its stiffness and buckling strength over that of a solid structure of similar weight by increasing its second moment of area. These characteristics should also increase the tensile strength of this skeletal component and increase its usefulness as a strong, stiff element in most echinoderm skeletons (Emlet 1982).

An analogous state of affairs obtains with the spines that stick out of the urchin's shell. Some of these are very large and are used by the animal to wedge itself into crevices of the coral reef on which it lives, so that it can resist the power of storm waves. The spines therefore have to accommodate damage and still be stiff and strong (figure 6.36). Because they have hierarchical fracture control, they can fail "gracefully." The spines are highly cellular, like an open-cell foam (sterome), with cells between 10 and 30 μm in diameter; porosity is graded from almost fully dense at the base of the spine to 60% farther up. At the nanometer level, the material is a highly ordered polycrystalline mesocrystal with conchoidal fracture behavior. The slight misalignment of the individual domains and the partially organic interphase (Oaki and Imai 2006) deflect cracks, increasing toughness. At the micrometer scale the high porosity of the sterome makes it light but weak, with thin crosspieces as predetermined breaking points that induce initial fracture perpendicular to the direction of compression and prevent crack propagation. The porosity also acts against propagation, and, as John Currey once pointed out, the extreme smoothness of the surface of the sterome, which is continually reworked by the cells that invest it, probably inhibits stress-concentrating effects and starter cracks. Thus a brittle material is turned into a crumple zone material (Presser et al. 2009). These two effects are common to the basic structure of the sea urchin skeleton, but the spines vary greatly in their macrostructure, which often has many separate structures such as ribs running along the outside. Again, these have a structural function, but their separation thwarts propagation of cracks. These three levels of fracture control enable an essentially brittle material to resist damage to a surprising extent.

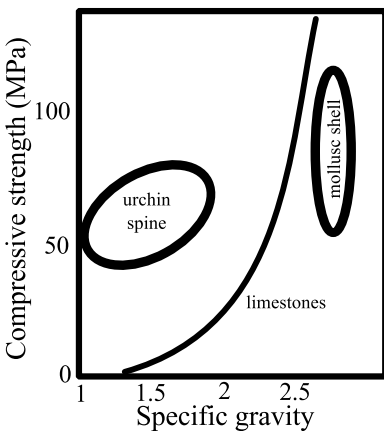


Figure 6.36. Comparison of strength of spines of sea urchins and of mollusc shell, compared with the mineral from which they are made (Weber 1969).

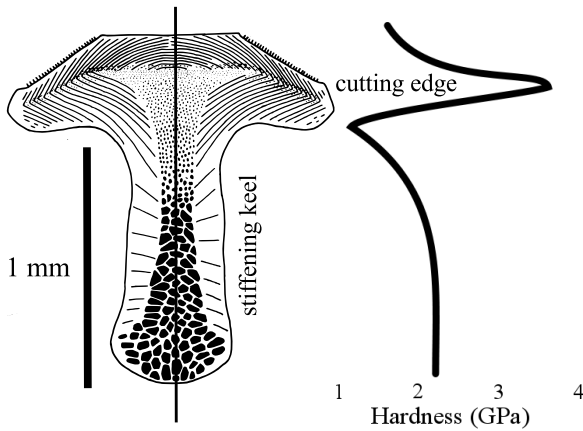


Figure 6.37. Indentation hardness profile (Wang et al.1997) through the section of the tooth of a regular sea urchin (Maerker et al. 1977). Lines and dots are sections through plates and fibers of dolomite in a calcitic matrix.

Urchin teeth (of which there are five) are slung in a frame (“Aristotle’s lantern”) from which they can scrape the surface (seaweed, rock) on which the animal sits. The tooth is basically a beam of a variety of cross sections; it is commonly a T shape with its cutting edge developed from a hard zone (stone area) within the crosspiece; the vertical part or keel serves to stiffen the tooth against bending (figure 6.37). The stone part is an oriented composite of dolomite fibers and plates in a calcite matrix (it is not known whether fiber and matrix contain different Ca:Mg ratios, which might be expected) with fibers $2\text{ }\mu\text{m}$ in diameter and a critical transfer length calculated as $16\text{--}18\text{ }\mu\text{m}$. The crystalline structure still appears (when studied by X-ray diffraction and polarized light) considerably simpler than that revealed by fracture. The hardness changes across the section of the tooth (Maerker, Kubanek, and Willgallis 1971) and along the section from the leading edge, through the stone area, and along the keel (Wang, Addadi, and Weiner 1997). The reduced hardness behind the leading edge of the working tip produces a self-sharpening system similar to that of rodent incisors and locust mandibles (section 7.32). Using different starting materials but requiring a similar function, these animals have arrived at a very similar structure for their teeth—an example of evolutionary convergence.

Implementing Ideas Gleaned from Biology

A brief outline of the history of biomimetics has been attempted elsewhere (Vincent 2008; Vincent et al. 2006), but nowhere has it been mentioned that the numerous lures and disguises used by hunters of stag and salmon (among the eligible quarries) and scarecrows on farms should also be considered! These examples take biomimetics back thousands of years. Currently, the medical profession is bidding to transform medical biomechanics into biomimetics. This is a credible goal in the general area of prostheses (Wolfe 1961) and some replacement tissues such as scaffolds for colonization by the body's cells (Kanungo and Gibson 2009, 2010) but not in the production of devices such as stents, which may reinforce biological tissues but do not replace them. Artificial hearts fall somewhere in the middle of this range and, if genetic algorithms are accepted as biomimetic, electronic cochlear implants do so as well. None of these are considered here.

In general, the current version of biomimetics is concerned with the interface between biology and other sciences and technologies. But we have to realize that biology is very different from other sciences, because the objects it deals with are complex and responsive and basically unquantifiable with our present methods. Organisms are the result of a process of serial and convergent solution of problems of survival through natural selection—their history is unknown. Thus whereas an engineer or physicist can be given a problem and has to find an answer, a biologist is given an answer (i.e., an organism that has evolved systems that ensured its survival) and has to deduce what the question was. And since most answers in biology are themselves an expression of several simultaneous functions, the “question” (which may be associated with the selective advantage of a particular trait out of several related ones displayed by the organism) may not be obvious. That's the “bio-,” but what about the “-mimetics”? Copying of one sort or another occurs quite frequently in biology and may be classified under camouflage (the organism looks, smells, sounds, or behaves like another object and so escapes detection) or mimicry (the organism takes on the appearance, smell, or sound of another organism to gain some advantage). Both forms of imitation are related to convergent evolution, the process by which two or more unrelated organisms develop the same or very similar solutions to a problem. Thus compound eyes have evolved at least twice, independently, in the phylum Arthropoda (Nilsson and Kelber 2007), and the camera eye has evolved independently in cephalopods and vertebrates—identical except for the direction in which the nerves attach to the retina—and in more generalized form in at least five other groups of animals (Morris 2006). This idea of evolutionary convergence as an

approach to biomimetics and to the solution of problems has some validity. In other contexts it might be called *analogy*.

7.1 Biomimetic Products

Living organisms are the mechanisms least like the technology on which we rely so heavily, yet they are being hailed as a major untapped resource of solutions to problems ranging from keeping dry in the rain to our survival on planet Earth. The resource is realized through technology transfer. The following list is not confined to materials, although they predominate. Neither is it exhaustive.

7.1.1 CAT'S-EYE REFLECTORS

When the tramlines were removed from the roads on which he drove, Percy Shaw realized that he'd been guided at night by the reflection of street lamps from their worn and shiny surface. However, he saw that the eyes of animals that are active in low-light conditions reflect incident light ("eyeshine") owing to the tapetum lucidum, a reflective layer at the back of the retina, which maximizes the light available for sight. In 1934 he patented a lens/reflector system mounted in a sprung stud in the road (figure 7.1), and the following year he founded Reflecting Roadstuds Limited to manufacture the system. The reflecting lens had actually been invented six years earlier for use in advertising signs by Richard Murray, and, as Shaw acknowledged, this invention had contributed to his idea.

7.1.2 VELCRO

Velcro is often thought of as the archetypal biomimetic product (Velcro 1955). The story about Georges de Mestral, with his hairy dog covered in clinging burr seeds after a walk through the woods sometime in 1948, is classic. De Mestral saw that the burrs, with their hooked tips, represented a temporary attachment mechanism. The driver for his inventive insight seems to have been a problem with his wife's zipper a

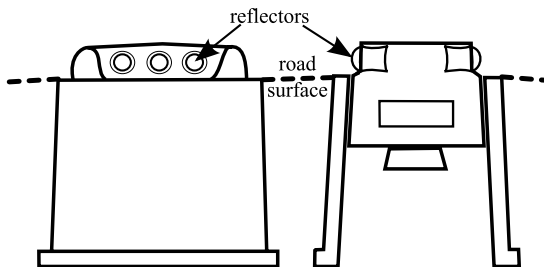


Figure 7.1. A prototype Cat's-Eye.

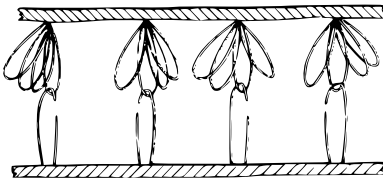


Figure 7.2. Prototype Velcro.

few nights previously (Petroski 1996). The transfer of this idea to a technical product was not obvious. After abortive trials over a period of several years, de Mestral found that the relatively recently invented nylon—itsself a biomimetic product, produced in imitation of silk (Viswanathan 2010)—was stiff and resilient enough to form a textile with molded hooks that could hold onto a furry substrate (figure 7.2). However, he had to overcome the additional problems of forming the loops in a textile, cutting them in the right place to make a functional hook, and cross-linking the nylon in infrared light to stiffen the hook. Finally, de Mestral had to find someone sufficiently interested in the idea to produce it.

7.1.3 ANTIREFLECTIVE SURFACES

Surfaces that gather light rather than dispersing it have been discovered on insect eyes (Bernhard, Miller, and Moeller 1965; Meyer-Rochow and Stringer 1993; Parker, McKenzie, and Ah Yong 1998) and insect wings (Stoddart et al. 2006). The structure can be nipples or ridges 200–250 nm high, spaced at the same periodicity. Ridges have also been found on the surface of epidermal cells of dahlia leaves (Koch, Bhushan, and Barthlott 2009), although they were not commented on. Nipples or ridges provide a functional gradient of refractive index, so that the reflective surface essentially disappears (Abbott and Gaskell 2007). This type of surface has now been manufactured on polythene sheet, which is then adhered to the glass surface of a solar panel (using glue that matches the refractive indices). The result is a significant improvement in capturing light. Abbott and Gaskell present interesting critical discussions on the mechanisms behind many of the surface effects currently subject to biomimicry.

7.1.4 LOTUSAN

The observation that the leaves of the lotus are always clean, despite its growing in muddy and stagnant water, led to the invention of Lotusan, a paint for making surfaces “self-cleaning” when wetted (Barthlott and Neinhuis 1997). Particles deposited on the treated surface are easily removed by water at normal pressures, such as rain. The effect is due to a combination of two factors: controlled surface roughness at the micrometer scale, such that any object makes contact with the surface only at the tips of the asperities, and hydrophobicity at the nanometer level due to wax crystals (or an amorphous coating of hydrophobic material). Similar surface textures have been observed in many other plants and in other systems, such as insect wings (Wagner,

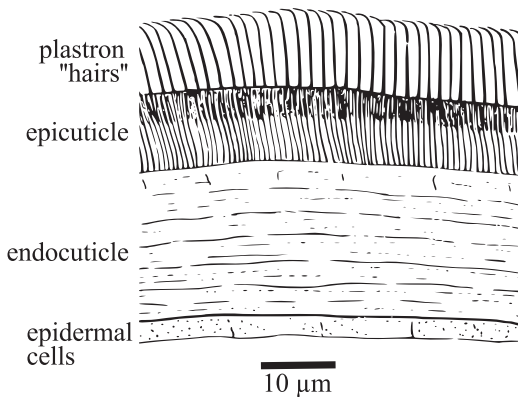


Figure 7.3. Arrangement of a plastron on the surface of an insect.

Neinhuis, and Barthlott 1996). The same surface properties have been developed on metals and various types of clothing, where they provide structural waterproofing.

This sort of surface property has been known for a long time (Cassie and Baxter 1944). The insect plastron (figure 7.3), a layer of air entrapped by “hairs” a few micrometers long arranged at a density of 10^7 or more per square centimeter, falls into the same general category of highly textured surfaces (Thorpe and Crisp 1947). The plastron is a well-researched system for repelling water; the thin layer of air provides an air–water interface at the tips of the hairs across which exchange of gases can occur, thus giving the animal a form of gill and enabling it to breathe under water. This is not only a common adaptation of insects living in, on, and around water but is obviously also a technique for keeping the immediate skin of the animal dry (since water cannot penetrate between the hairs) and thermally insulated. The sportswear company Finisterre in the United Kingdom developed a successful breathable and warm waterproof textile for surfers that is rather like a velvet. Its inventor, Tom Podkolinsky, did not know about the plastron when he developed the material, although he based his initial concepts on the waterproof pelage of seals and otters. Another technical version of the same concept is an extremely water-repellent foam whose surface allows direct extraction of oxygen from aerated water (Shirtcliffe et al. 2006). Water-repellent surfaces are also being developed to make devices that can walk on water, using design ideas from *Gerris*, the pond skater.

7.1.5 BIOMIMETIC WOOD

Possibly the only fibrous composite that can properly be called biomimetic is a wood analog (Gordon and Jeronimidis 1980). The starting question in 1975 was, How do we account for the high work of fracture of wood? The model current at that time was simple fiber pullout, which was shown not to account for the high energy absorption. The thickest wall of a wood cell in a softwood such as spruce, about 80% of the thickness, is called the S2 layer (figure 5.27), in which the cellulose is oriented in a single direction and winds around the cell at about 15° to the longitudinal axis. A single wood cell is essentially a long tube, rather like a drinking straw. If a tubular

model made with fibers wound helically around it at 15° is broken in tension, it partly buckles inward and partly develops a helical fracture between the fibers that travels along the tube some distance before stopping (figure 7.4) when it runs out of strain energy. Then, much like in the fracture process of the *Conus* shell described previously, another crack starts and travels until it, too, is exhausted. This structure not only provides toughness, owing to the multiplicity of cracks, but also exhibits a force-deflection behavior like that of a metal with a distinct yield point and a post-yield region where the material, although having failed, is still capable of supporting a load (figure 7.5). This type of failure has been observed in wood, in which the individual cells additionally pull apart laterally as they buckle inward, absorbing even more energy (figure 7.6). But although the helical organization of the cellulose fibers increases toughness, it reduces the axial stiffness of the cell. When the two halves of the piece of wood finally part company, the helical fractures leave sharp-ended splinters. An interesting point about this toughening mechanism is that it is formed from two brittle materials, and so it is the structure that confers toughness.

Early forms of the models were assemblages of helically wound tubes, but these were not very easy to wind and glue together, although weight for weight the resulting wood analog was five times tougher than any other man-made material (table 7.1). There is a cheaper and easier way to make the models, although the energy absorption is not so good. Sheets of uniaxial prepreg, in which the fibers are oriented in a single direction and embedded in uncured resin, are folded to produce a corrugated structure (Chaplin, Gordon, and Jeronimidis 1983). The fibers are arranged at an angle to the long gaps between the corrugations (figure 7.7). This material was developed

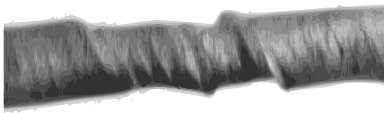


Figure 7.4. A helically wound tube of glass fiber in a plastic resin. The diameter of the tube has been exaggerated by a factor of about 10 to emphasize the places where the tube has broken. The original winding angle of the fiber was 15° to the long axis (Gordon and Jeronimidis 1980).

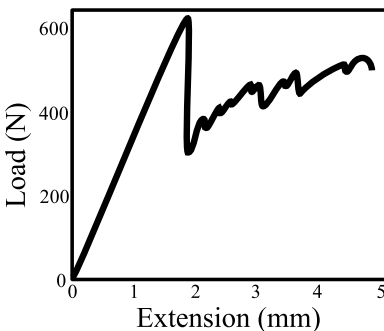


Figure 7.5. Load-deflection curve for a tube like the one in figure 7.4 (Gordon and Jeronimidis 1980).

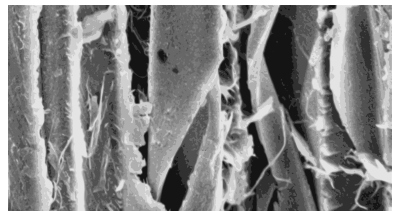


Figure 7.6. Part of a piece of spruce wood broken in tension, along the length of the cells (Gordon and Jeronimidis 1980).

TABLE 7.1
Properties of wood analog compared with standard materials

<i>Material</i>	<i>Specific gravity (GPa)</i>	<i>Stiffness</i>	<i>Specific stiffness</i>	<i>Toughness (kJ/m²)</i>	<i>Specific toughness</i>
Carbon-fiber-reinforced polymer	1.6	140	87.5	1	0.6
Oak	0.68	10	15	7	10
Analog, corrugated	0.61	11	18	10	33
Tough aluminum	2.7	70	26	400	150
Tough steel	7.8	206	26	1220	156
Analog, tubes	0.65	11	17	500	820

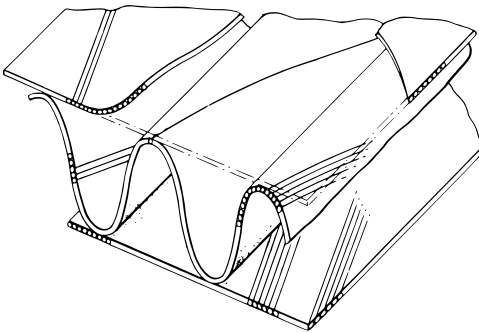


Figure 7.7. A corrugated form of wood analog made of three layers of uniaxial fibrous sheet (Chaplin et al. 1983).

for protection against explosives, knives, and bullets. It is particularly well suited to use in clothing, since it is so light, being a cellular material. In a series of tests in which bulletlike projectiles were fired into this wood analog, its in-plane compressive strength was hardly compromised, whereas a solid carbon fiber-reinforced plastic sheet retained less than 40% of its strength. In a design study using the wood analog as a riot shield, the cost increased about 25%, but the weight of the shield was halved.

7.1.6 NACRE

Mother-of-pearl is a deceptively simple material—a layered material rather like the bricks in a wall—yet very few people have succeeded in making a credible biomimetic version. Bill Clegg, a professor of materials at Cambridge University, needed to make a toughened ceramic. But such ceramics are difficult to make, and expensive. Clegg developed a ceramic with superior heat resistance and toughness that he used to make the combustor liner for a gas turbine. The nacre mimic was made of sheets of silicon carbide 200 μm thick doped with boron (which aids densification), with graphite powder between the layers, partly to separate them and partly to allow them to move relative to one another. The sheets were assembled and sintered. The graphite powder that separates the layers makes it difficult for a crack to move between layers and also allows the material to expand and contract freely. Although Bill didn't realize it, the concept of matrix as lubricant—which is alien to the current concept of composites—seems to be an essential part of the design of nacre (Barthelat

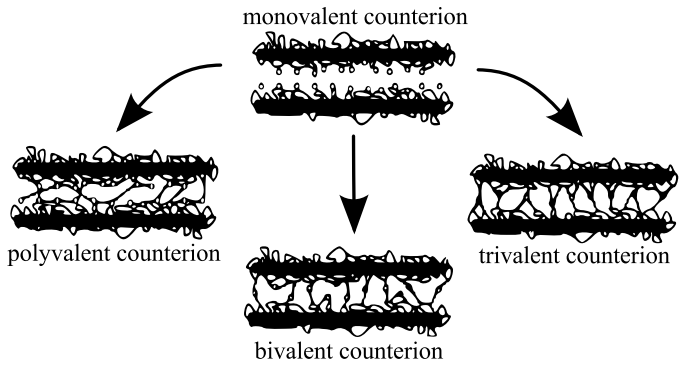


Figure 7.8. Cross-linking between clay particles depending on the valency of the added polymer (Walther et al. 2010).

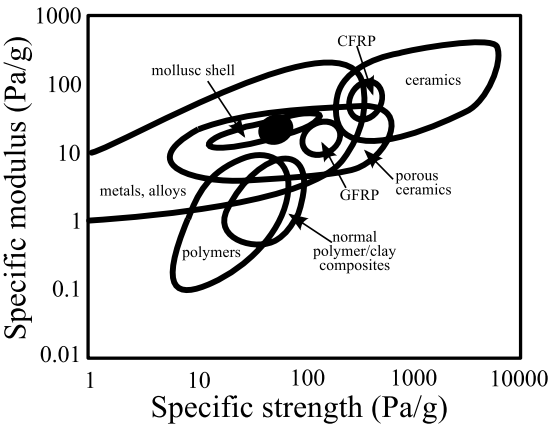


Figure 7.9. Strength–stiffness of a number of materials: the “nacre paper” is the large black spot near the center (Walther et al. 2010).

et al. 2007). More recently, Walther et al. (2010) made a nacre mimic using clay particles in a polymeric matrix (figure 7.8), employing the technology of papermaking to drive the assembly process (Walther et al. 2010). The matrix can have different degrees of cross-linking depending on the chemistry. This material has outstanding properties in tension (figure 7.9); the Young’s modulus and stress at break can reach 40–70 GPa and 80–135 MPa, respectively. The material is remarkably tough under wet conditions. Dynamic processes, such as sacrificial (dynamic) bonds and hidden-length scales contribute significantly to toughness and to the ability of the material to repair itself, just like real nacre.

7.1.7 CRAIG’S ROOF

Salmaan Craig, while he was a student with the engineering firm Burow Happold, attacked the problem that buildings in hot areas can get uncomfortably hot during the day. To reduce heat gain, the buildings are insulated, but this prevents their losing heat

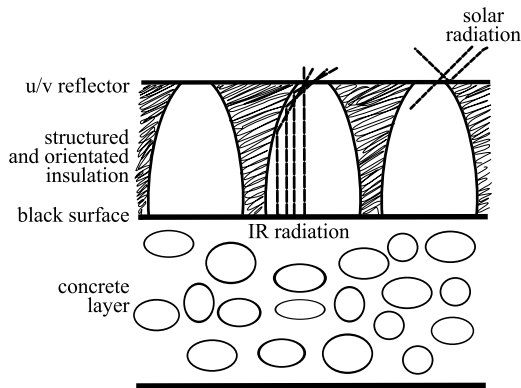


Figure 7.10. Craig's Roof (second version).

to the night sky, the best way of cooling them. Craig developed a roofing system in which the insulation keeps out heat from the sun (shortwave radiation) during the day but provides a route for long-wave radiation to exit the building during the night (Craig et al. 2008). In his design, the concrete of the roof stores heat during the day, and the insulation is structured with an orientation to provide an exit pathway for radiation during the night (figure 7.10). Although there is no indication of its derivation, this system can be classified as a biomimetic solution to the problem, since it evolved from the suggestion that the strongest design variable at the size level of roof insulation is “structure.” The engineering answer is “energy,” and, indeed, most people would see the solution to an overheated building as an air conditioner. This answer would be acceptable only if the air conditioner were driven by solar energy via photovoltaic cells!

7.2 Quasi-Biomimetic Products

There is a strange scientific hinterland of successful products whose design is based on a misunderstanding of how the relevant biological system works. This need not be a negative factor; as long as the technology works properly, there is no problem. There are two interesting outcomes of this apparent failure: the possibility that the design will work better when the “proper” version of the biological mechanism is adapted, and the possibility that the newly developed technology will stimulate further research in biology to see whether the phenomenon actually does occur.

7.2.1 TEXTILES

The hairs of the pelt of the polar bear are supposed to be light guides. A textile based on this concept has been produced at ITV-Denkendorf, the German textile research institute. These hairs most definitely are not light guides (Koon 1998), but the people at ITV seem to be in denial when they say, “So, the fur is not only camouflage but

also a light trap,” having quoted Koon’s paper, which specifically disproves this notion (Stegmaier, Lilinke, and Planck 2009). Such a statement isn’t necessary to show that a textile with a built-in light guide can serve useful functions, and there seems to be little point in trying to smuggle “green” credentials past a critical audience.

7.2.2 ARCHITECTURE

The interplay between biology and design—especially architecture—has a long history. Most of the design has been decorative and has been called “biomorphic” or “zoomorphic” (Aldersey-Williams 2003). Such shapes can be immensely appealing but rarely have functional advantage. Biomorphism can also give rise to misleading stories. For instance, the claim that the leaves of the lily *Victoria amazonica* inspired Joseph Paxton in his design of the corrugated roof of the Crystal Palace is wrong. The roof was invented by horticultural journalist and revolutionary John Claudius Loudon sometime in the first decade of the nineteenth century (Colquhoun 2004). The “ridge and furrow” glass construction maximized the entry of light and heat to the greenhouse, particularly in the early morning and late evening, when the sun is low in the sky. It seems quite probable that Paxton used the leaf of *V. amazonica* (which he grew in his greenhouse in 1849) to suggest the design of the end walls of the great central arch atop the building (figure 7.11), but this is an architectural element and doesn’t appear on Paxton’s famous sketch of the original design made on a piece of blotting paper! Thus the Crystal Palace had biomorphic elements but was certainly not biomimetic.

Similar stories have grown up about the Eiffel Tower. It owes nothing to biology. The original design came from Maurice Koechlin and Émile Nouguier, two junior engineers who performed the preliminary calculations. The curvature of the uprights was calculated for least wind resistance (Anon 2010). Stories that the design had

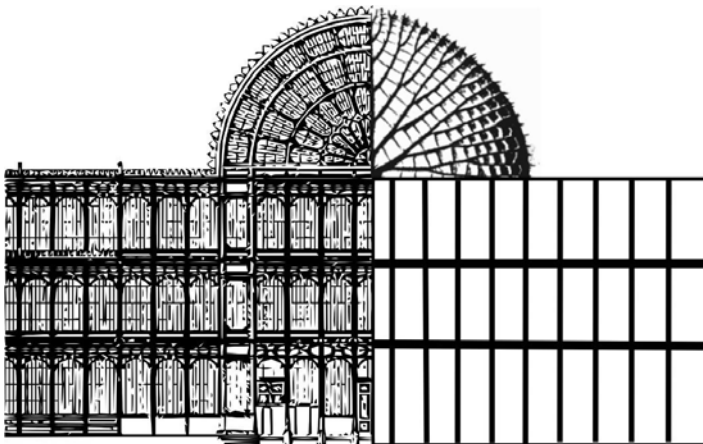


Figure 7.11. Front elevation of the center of the Crystal Palace, showing how the iconic leaf of *Victoria amazonica* might have contributed to the design (right half).

anything to do with the arrangement of the trabeculae (small bony supports) in the head of the human femur are laughable.

The Eastgate Centre in Harare, Zimbabwe, depends for part of its cooling on passive flow of the sort that occurs up chimneys when there is no fire, owing to the difference in wind speed at the top and bottom of the chimney: the higher wind speed at greater height leads to lower pressure (the Bernoulli effect), so air is sucked up the chimney, to be replaced by air from ground level. This form of cooling buildings was in use in Persia at least 2000 years ago. However, the mistaken (although contemporaneous) idea that termites use the same system led to a design whose origins may have been biomimetic, but only in concept rather than reality. Termites do not significantly control the internal temperature of their mound; they rely far more on the thermal mass of the soil from which the nest is made. Many mounds do not vent to the outside, and even those that do are too short to be able to capitalize on the velocity gradient of the wind (Turner and Soar 2008). The Eastgate Centre cannot rely on passive ventilation—it needs quite powerful fans to be effective. Indeed, the time when cooling is most needed—when the weather is hot—is the time when winds are least prevalent!

7.3 Techniques for Biomimetics

So far, most biomimetics has resulted from developing the technical potential of biological mechanisms discovered in a more-or-less adventitious manner. The understanding of design is important for biomimetics, since it is biological “design” concepts that are being recognized and transferred to technology. There are a few books on design in engineering; for a complete and masterful overview of all aspects of design, in all areas (don’t believe the restriction implied by the title), you should read Pahl and Beitz’s *Engineering Design: A Systematic Approach* (1996). The more specialized area concerning the transfer of “design” from one discipline to another has been given the generalized name *design-by-analogy* and is developing into a widely used approach, independent of biomimetics (Verhaegen et al. 2011). Books and papers exploring this area present a variety of algorithms, with actions and questions arranged so that the problem or the design is developed as progress is made. The steps in the algorithm can be arranged as a ladder, maze, spiral, helix, or line. Many of these are rather trivial and are based on the practice of a particular company, so do not become entrapped by the protestations of “The Only Way” or “The Design Spiral/Helix/Ladder.” However, some of the algorithms are objectively designed, using ideas from artificial intelligence, cognition, or a well-established technique such as TRIZ.

7.3.1 DATABASES

The most obvious method, and increasingly the least effective, is to make a list of what is available. Any list has to be capable of being interrogated and sorted in various ways. An attempt at the University of Bath (Bogatyreva, Pahl, and Vincent 2002;

Vincent et al. 2005) foundered for a number of reasons, mostly to do with the complexity of the data needed for a complete description of each biological example and the associated difficulties in coding from scratch. Indeed, a quick search of the Internet shows a number of databases that have disappeared without a trace or have been started but have seized up (Le 2010). One difficulty with these databases concerns the multifunctionality of biological systems. Which function should be represented? To what depth of detail is it useful to describe each biological example? It's difficult to imagine how Velcro could have been invented if de Mestral had relied on a simple database. The burdock plant isn't interested in zippers; it needs to spread its seeds. If these seeds can be large and heavy, they will have a better chance of surviving and germinating; and if someone else can carry them, the plant will need to expend less energy in a distribution mechanism. These requirements are all very different from those necessary for a zipper substitute. As a test—how many biological databases would list transport in an animal's gut as a possible biomimetic mechanism? But it is important to the seed, for both its travel and ease of germination.

Biology and engineering generate different expectations. Organisms evolve by the interaction of variation and natural selection; biology is largely descriptive and creates classifications. In contrast, engineering is a result of making decisions; it is prescriptive and generates rules and standards. In an interdisciplinary world there is sometimes a tendency among engineers to expect that they can generate rules about biology, whether or not such rules do, or even can, exist. Biologists tend to start with the organisms and see what the organisms can do, and then group and classify them according to function. But they rarely go further. An example is the Biomimicry Institute database "AskNature" (Various 2009), which has gained great currency. Beautifully prepared and presented, it suffers from the basic problem that although it addresses specific topic areas related to technical problems, the database makes little attempt to analyze the problem that the organism is solving, although it recognizes and emphasizes the importance of function. It leaves it up to the person referring to the database to decide how useful each example is and what technical problems it might solve. We need more clues to point to biological mechanisms that might be useful.

There is no doubt that "function" has to be a primary filter, in that technical problems can be expressed in terms of the function required (control temperature, detect movement, etc.), and biologists have arranged organisms in this way for many years. This comparative approach highlights the convergence of organisms onto relatively few solutions offering survival. It's the bane of taxonomists, since to an individual its survival is of far greater importance than its phylogeny! Thus taxonomy commonly relies on the use of features that are shielded from natural selection, such as the number and arrangement of hairs in a noncritical part of the organism. However, convergence can be of distinct advantage to the biomimeticist. Biologists can generally interpret the mechanical and chemical aspects of an organism only in terms of the physics and chemistry that is known and can be applied. But if organisms regularly exhibit a convergent trait for which there is no obvious explanation in technical terms, then obviously, the organisms are using some aspect of physics or chemistry that we do not appreciate. Why, for instance, do so many arthropods (insects, spiders, pycnogonids, etc.) have long, thin legs when these are not seen in other phyla? The

advantage is not obvious (a long thin strut is much more liable to buckle and bend), but it is obviously there. This lack of understanding highlights a major problem—biomimetics commonly applies to technology only those concepts that technology can recognize, since the recognition pattern was itself created by technology. Convergence (= analogy) may be the only way out of this procedural dilemma.

7.3.2 ONTOLOGIES

An ontology is far better than a database for this sort of application. One that is readily available and free is Protégé, based on OWL, the language used to create the World Wide Web (www). Information in Protégé is held in a hierarchy of classes and subclasses, which are defined by the user. A class can contain any number of subclasses at the same level, and the hierarchy of sub-, sub-sub-, etc., classes can go to great depth, although it is not always desirable to do so. In this respect an ontology is exactly like a biological taxonomy. Classes are given suitable names, and their members are related to one another by a range of appropriately chosen properties. These properties can relate objects to one another (Object Properties), or they can confer specific data quantities onto objects (Data Properties). Additions and changes can be made on the fly, so that the size, range, and complexity of the ontology are increased or decreased as required. Of course, this structure is very different from that of a conventional database. Within broad limits an ontology can be greatly modified at all stages of its use and development. This flexibility is particularly convenient, because the temptation is always to include too much information at the outset, since one is never sure what information is necessary, sufficient, or significant in any other way. In Protégé the redundant information can easily be deleted or reformulated.

The heart of the ontology is the establishment of relationships between classes and their members. Crucially, the reasoning is based on an “open world” model as opposed to “closed world,” which is the case with most databases. In a closed world assumption, if something is not stated to exist, then it is assumed not to exist. However, in an open world model, as used by OWL, if something is not stated to exist, then it is impossible to say whether or not it does exist. A clear statement as to its existence or nonexistence has to be made. Therefore, in Protégé it is important to state everything explicitly.

Large parts of ontology rely on set theory. Classes can be defined as disjunct (i.e., nonoverlapping) or intersecting (overlapping sectors). Properties can relate a pair of items (e.g., father to son) or encapsulate more complex relationships such as reflexivity (e.g., two siblings, who therefore have the same relationship with each other) or transitivity (e.g., any friend of yours is a friend of mine) and also can be given rules about how many members there should be in a single class (cardinality). Thus a range of rules of relatedness is used to generate a web of interrelations and properties that is far more complex than a database. This web can then be interrogated. The interrogation can take several forms. A simple command would be “list the items or classes that have this particular attribute.” A number of restrictions can also be chained together, using the relations “some,” “only,” “and,” “or,” and “not.” In this way a concept can be derived that is implicit in the available data but that may not be obvious.

An ontology can be used in many ways. Two examples in biomimetics are given here. The first is based on a list of classes taken from the “Conflict Matrix” of TRIZ. TRIZ (*Teorija Reshenija Izobretatel'skih Zadach*), which translates most accurately as the Theory of Solving Problems Inventively, is a system masterminded by Genrich Altshuller and developed in Russia in the 1950s and introduced to the Western world in the early 1990s. Its prime assumption is that most technical problems have been solved and that a suitably coded problem statement will always yield a good answer, frequently a novel one. In its current form, TRIZ is populated by data generated by the analysis of several million patents. Altshuller found that a problem can often be characterized as a pair of factors, one of which is the factor that is to be improved (the thesis of Hegel's philosophy) and the other, any perceived disadvantage of implementing the improvement (Hegel's antithesis). This pair of factors, selected in a matrix of such factors, is used to identify one or more “Inventive Principles” that have been shown by their presence in “strong” patents to solve the problem. These principles are also available in a look-up table. There are 39 factors and 40 principles, but these quantities are not implied by any assumptions or requirements, so there is no theoretical basis for the system—it is completely pragmatic. Thus TRIZ can be extended if it is found to be inadequate to describe biological problems and their solution. The technique involves interpreting the adaptations an animal or plant has made to a particular environmental problem in terms of the Inventive Principles of TRIZ by taking the information from published research papers (equivalent to a patent claim). Because the Inventive Principles are all transformative functions, they are independent of the context within which they are to be used, which can therefore equally be biology or technology.

The results summarized here were selected from the analysis of about 300 scientific reports of specific problems and their solution by organisms. For example, insects that feed on plants need to keep their mandibles sharp. They do this partly by incorporating Zn or Mn into the cuticle, which hardens a capping layer, but also by allowing parts of the mandible to break away from beneath the capping layer (Hillerton and Vincent 1982), much like the renewable blade tip of a hobby knife is broken off. Similarly, the chorioamnion needs to fracture in a controlled fashion (Calvin and Oyen 2007), as does the stigma of some plants after pollination (Y. Heslop-Harrison and J. Heslop-Harrison 1997), and the autotomizing leg of an escaping spider (Amaya, Klawinski, and Formanowicz 1998). Here we have a function in common among a plant, two arthropods, and a mammal. The connection illustrated was made by independently assessing of each of these four examples and assigning to them the appropriate TRIZ Inventive Principles (within the large rectangle of figure 7.12), which were remarkably coincident. The three Principles in the inner rectangle have a pair of factors (the Hegelian thesis and antithesis) of reduced strength (the line of weakness along which the object breaks) with the advantage of greater longevity of the organism. The fourth Principle, outside the inner rectangle, emerged from the analysis but was not provided by the Conflict Matrix. It is therefore an additional (biomimetic) design concept. Another example compares the wings of insects (Combes and Daniel 2003) and eagles (Carruthers, Thomas, and Taylor 2007) and the way they deform elastically as they are flapped. In this instance there are

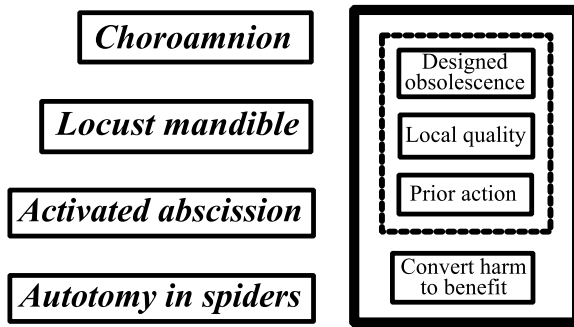


Figure 7.12. Comparison of fracture occurring in natural systems (left) and design concepts used (right). There is total connectedness between the boxes on the left and those on the right.

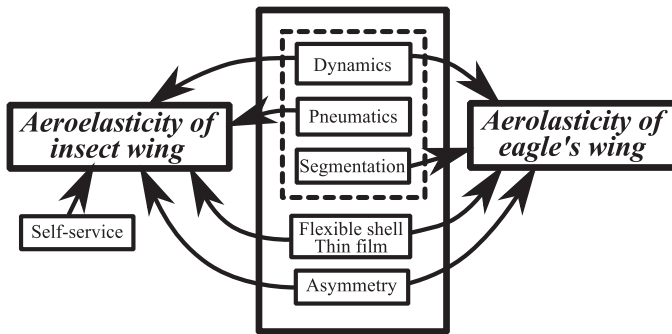


Figure 7.13. Comparison of aerolastic effects in insect and bird wings. Arrows show applicability of the design concepts. The concepts surrounded by a dashed line are suggested by TRIZ; the rest are derived from an ontological analysis.

two relevant Inventive Principles that TRIZ does not predict and one that is unique to insect wings, which are not reliant on intrinsic muscles in the way that an eagle's wings are (figure 7.13). Their aerolastic behavior is much more due to the way their structure responds passively to the changing air pressure as the wing flaps.

The second example of an ontology is taken from the Georgia Institute of Technology, Atlanta, where Ashok Goel is heading a project that is developing an ontology for biomimetics (DANE—the acronym for Design by Analogy to Nature Engine—available at <http://dilab.cc.gatech.edu/dane/>) but taking a very different (i.e., informed!) approach based on a Structure–Behavior–Function ontology, developed by the GTech Design Intelligence Laboratory over many years. “Structure” concerns the “what” of a system—the elements, components and materials, and their properties. “Behavior” has to do with the “how” of a system—the states of the system (e.g., on or off) and the means by which the state can be changed. “Function” can be summarized as “A thing/does/something/under certain conditions” (i.e., subject/verb/object/

conditions). (This construct is also used in TRIZ as a ploy to escape “psychological inertia”—in modern parlance, the tendency not to think outside the box). Such a statement can be made with no implied context and so can form a bridge between different technologies and biology. At present DANE is populated by biological examples that describe the components of organisms and the way they work, and it’s possible (for instance) to interrogate it to find out how water can be transported by different organisms in different ways. However, whereas DANE presents ways in which a particular outcome can be achieved (e.g., desalination of water [Swaroop, Helms, and Goel 2010]), the TRIZ-based system shows how a particular transformation can be achieved (e.g., the characteristics necessary to produce an aerolastic wing). This difference is simply due to the diverse approaches to defining what is required to do biomimetics. We are at a stage where we do not know the classes of problems, the stages of processing, or the conditions of knowledge under which different methods/tools are more/less useful for biomimetic design. However, ontologies currently available are, in general, hampered by their small size, because the data are assembled and analyzed by hand. This process is tedious and difficult, and there is no guarantee that the data will be useful in solving problems, although an ontology of biology can be a fascinating system. We need to develop a more automated approach, such as that used in the creation of the Gene Ontology.

7.3.3 LEXICOGRAPHY

An ontology is assembled from natural language by arranging verbs and nouns in various logical ways within the context of a program. One of the main problems is keeping the ontology focused, especially when the final use of the ontology is not certain—What particular problems will it be asked to solve? Another method uses one or more biological texts as a resource assumed to be “complete” (or at least relevant) and then employs lexicographic methods to search for appropriate words and hence appropriate organisms or functions. This is the approach taken by Li Shu at Toronto University (Shu 2010). Two major problems here are that biologists and engineers use words differently (“strain” is an example—setting aside the fact that few biologists know the difference between stress and strain—“strain” can also apply to a particular breeding line), and a large part of the project is to explore biological concepts and mechanisms that are unlike those commonly met in technology. It is therefore crucial to have a loop built into the exercise so that the functional definition can be repeated and refined. An example used by Shu is the engineering concept of “clean.” In biology a “clean” organism can be considered as one without detrimental pathogens, and cleaning or cleansing can be thought of as defending the organism against an invader. Thus “defend” becomes a valid alternative to “clean” and can open up new lines of analogy. But the two words are not accepted as synonyms, so Shu developed a technique she called a bridging mechanism (figure 7.14) that identifies nonobvious, but relevant, words used by biologists (Chiu and Shu 2005). A main target of the method is to extract biological keywords objectively and repeatably without the assistance of a biologist. This target seems to have been achieved.

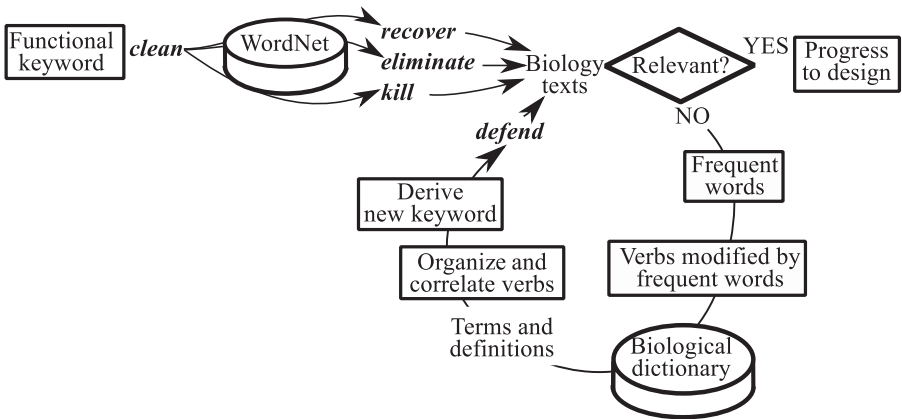


Figure 7.14. Flowchart for deriving suitable terms from biology to use in biomimetic design (Shu 2010).

7.3.4 AN IDEAL?

The big advantage of ontologies is that they are a repository of knowledge that can be expanded and questioned, and can intrinsically generate new ideas. An ontology of biomimetics generated by lexicographic methods that back up (and/or are integrated with) a problem-solver such as TRIZ, for which ontological programs are being developed, is the ideal. Descriptions of such systems exist, with files that can be downloaded (Bowers, Madin, and Schildhauer 2010; Cimiano et al. 2011; Liu, Hoga, and Crowley 2011; Santoso, Haw, and Abdul-Mehdi 2011), but this is a fast-growing area and will therefore not be covered.

7.4 Instead of Energy ...

One way around the complexity problem is to ignore it and categorize biological phenomena at a coarser level of detail. This is a valid and surprisingly successful approach, as it allows easier comparison of biology and technology and suggests design routes for rectifying some of the more damaging excesses of our current technology (Vincent et al. 2006). If we start with the mantra “Things do things somewhere” (compare with the previous definition of “Function” in DANE, there is no difference other than the “somewhere,” which is a different class of problem; convergence!), each of the three sections can be decomposed into two components. “Things” becomes Substance and Structure; “do things” is represented by Energy and Information; “somewhere” is in Time and Space. A large number of examples of problems in biology (about 2500, gleaned from the literature) and technology (about 5000, gleaned from the Internet) were categorized according to which of these six parameters best represented the main control variable that solved the problem and were assigned to a size ranging from molecular (nm) to civil engineering

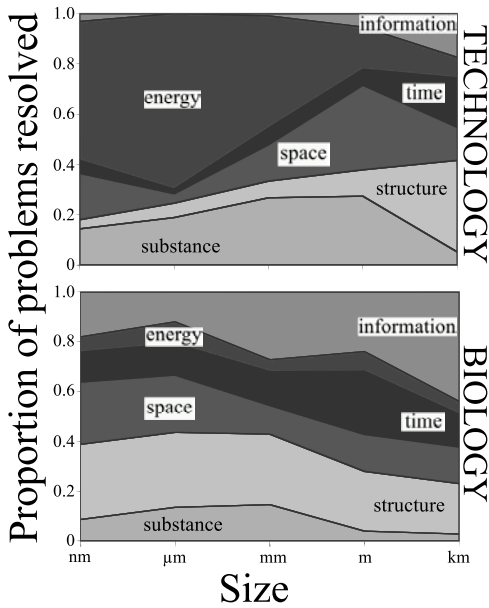


Figure 7.15. Different use of substance, structure, energy, space, time, and information in solving problems in biology and technology (Vincent et al. 2006).

or ecosystem (km). The two graphs in figure 7.15 show that our current technology relies heavily on the manipulation of energy for the solution of problems, especially for materials processing (at the micrometer level), where energy is used as the main variable to solve up to 75% of problems. By contrast, energy is the least-used controlling variable in biology (only 5% at any size) and is replaced (at the materials processing level) by information (15%) and structure (30%). At larger sizes, information becomes even more important in biology, appearing as pheromones, sound, and the like. These data are interesting, since although we have known for many years that our technology is too reliant on energy, we have not had a suitable indication of what might replace it. Biomimetics presented in this fashion, far from being a miscellaneous collection of techniques and ideas for incorporation into the current engineering paradigm, challenges the way in which engineering is carried out. In many respects it finds engineering wanting, not least in its extreme reliance on energy. This drawback was already known in a vague sort of way, but this analysis assigns values to the differences and suggests a way out. We can examine this situation more closely by counting how the 40 Inventive Principles are invoked in the solution of biological and TRIZ solutions to the same or similar problems (as defined by the thesis–antithesis pair) after grouping them into the six main fields just defined (Vincent, in press). The examples explored here in more detail are those that biology emphasizes. Therefore, if you have a problem, the following Inventive Principles will guide you to a more “biomimetic” solution, simply because these Principles are the most “biological.”

Notably, the only energy principle that biology uses to a significant degree (compared with nine used by technology) is “Prior counteraction” (figure 7.16). Examples

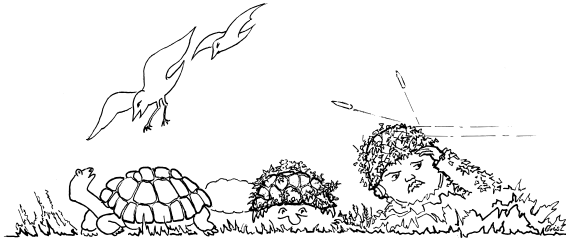


Figure 7.16. TRIZ Inventive Principle No. 9, “Prior counteraction” (© C Isaac).

follow. The cellulose in all nonwoody plants is prestressed in tension, which allows the amount of solid material in the structure to be minimized. The provision of protection against the physical environment is a general property of living things—extreme examples are penguin feathers (against cold) and thick bark in eucalyptus trees that can peel off as a sacrificial layer to protect against dust pollution, or be burned away, which protects the tree from fire. Technical recommendations would be to prestress a material in tension to allow the structure to take compressive forces, or to provide protection before the challenge and use an electric heater to preheat the car engine before starting (we shiver instead). In fracture mechanics there are many toughening mechanisms that are “hidden” within the material and not expressed until the structure starts to fail. Examples are microcracks and sacrificial layers.

7.5 . . . Use Space . . .

Among the Inventive Principles that fall under the heading of “Space” are “Asymmetry” and “Another Dimension.”

7.5.1 ASYMMETRY

The “Asymmetry” Principle suggests changing the shape of an object from symmetrical to asymmetrical or increasing its asymmetry (figure 7.17). Very few biological objects are symmetrical and so are difficult to model and analyze. The asymmetry is usually a direct response to the use or history of the object and so represents an immediately adaptive and efficient response to function. Symmetry in engineering is usually present only for ease of fabrication and calculation. An example is an electric furnace with electrodes placed asymmetrically to permit the continuous loading of ore on one side and the discharge of molten metal on the other. Asymmetrical paddles mix more effectively than symmetrical ones (both for concrete and for cake batter!), and asymmetrical scissors are easier on the hand than symmetrical ones. Mass customization is a business strategy that corresponds to asymmetry—the product or service or policies of a business are specifically designed for each customer and don’t need to be the same as those provided to other customers.

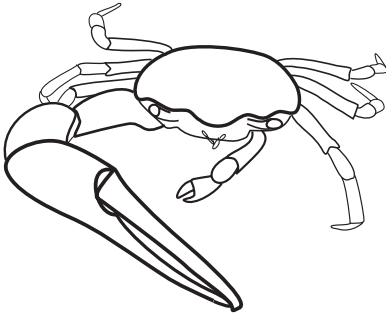


Figure 7.17. A rare example of asymmetry in nature—*Uca*, the fiddler crab.

7.5.2 ANOTHER DIMENSION

The “Another dimension” Principle uses an extra dimension of space or deployment (figure 7.18). Biological organisms make maximal use of the spaces available. Their limitation is usually mechanical rather than conceptual. Thus the more primitive organisms tend to align themselves with environmental surfaces because they lack the stiffness or stability to move away from them. Mosses grow on the ground or as low mounds; only higher plants have developed a stem of sufficient stiffness for the plant to raise itself off the substrate. The tendency, then, is for more advanced plants to grow taller and compete more effectively for access to sunlight and better dispersal of seeds.

In an artificial world, such as conventional gardening, plants are placed in rows, that is, “one-dimensionally.” Square gardening is a method developed for small gardens. Plants are placed in square blocks (a 1-m-square block subdivided into nine equal squares), that is, “two-dimensionally.” Plants are placed much closer than in large-scale agriculture, and the yield is higher, since weeds are almost nonexistent because of the close spacing.

Use of tunnels and underground buildings is increasing, and there are increasing numbers of tall buildings, multilevel highways, and overpasses. A multistory city is actually evolving. The engine in a car is angled so that in case of frontal impact, it slides beneath the car rather than into the passenger compartment.

Sometimes, the additional dimension is made invisible. Disney World in Florida pioneered the multidimensional concept now used in many amusement parks. A

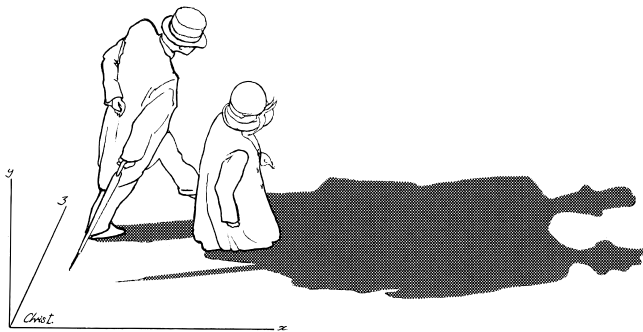


Figure 7.18. TRIZ Inventive Principle No. 17, “Another dimension” (© C Isaac).

network of tunnels, workshops, dressing rooms, storerooms, and staff centers runs under the park. A character is never seen in a part of the park that doesn't match his or her role—instead, the worker vanishes from one area and uses the underground system to travel to the new area, or to rest, or to remove or replace parts of a costume. This preserves the illusion of the character for the visitors. Less glamorously, garbage is dumped into another system of tunnels, so that the visitors never see garbage being transported through the park.

Information systems may store data in multidimensional arrays. One, two, or three dimensions may be visible to the customer (or to the customer service employee), and the rest of the structure is hidden but helps make the data available. Similarly, three-dimensional networks of business relationships respond faster to the needs of one member than one- and two-dimensional systems do. Wafers stacked on top of one another in three-dimensional integrated circuits greatly increase connectivity and power.

7.6 . . . or Use Information . . .

Biology depends on information (derived from the order of bases along the deoxyribonucleic acid molecule, and presumably from external influences when on a micrometer scale and greater) that ultimately (among other effects) defines the chemistry and hence conformation of the two main structural polymers of biology—proteins and polysaccharides. With the added morphological variables found in fibrous composites and cellular materials, biological organisms have evolved a remarkable range of properties that almost exactly match the specific stiffness and specific strength of technical materials (figure 7.19) and probably do far better with specific toughness. This “mix 'n' match” approach has been explored with technical materials (Ashby and Brechet 2003): its application to biological materials (figure 7.20) summarizes their composition.

It is disputable that the biological system inherited on this planet is the paradigm for sustainability, but there is no evidence that any other system would provide the resources needed for survival, at least on planet Earth. Several approaches were alluded to in earlier parts of this book. The starting point of a biomimetic approach would be to synthesize polymers that can assemble themselves into structures—liquid crystals—and this we can do to a limited extent. Liquid crystals are certainly the basis of the most highly performing natural materials, such as silks and cellulose. When the forces available for self-assembly become too weak because of increasing size of the assembled aggregates, then we should seek other resources. The most convenient, and the most easily controlled, are fields (gravity, air pressure, electromagnetic fields, etc.), which suggests that self-assembled components should be organized by precipitation or aggregation within a controlled field. This, too, happens in biology, where environmentally induced strains are a main driver for shape, size, and microstructure (Hepworth and Vincent 1999; Mattheck 1998; Patterson 1992). At the next size level we need to combine net forming and assembly techniques; the former is being developed with molding (a traditional technique) and rapid manufacture. Unfortunately, rapid manufacture does not utilize the possibilities of hierarchical construction, although it is possible immediately to think of ways

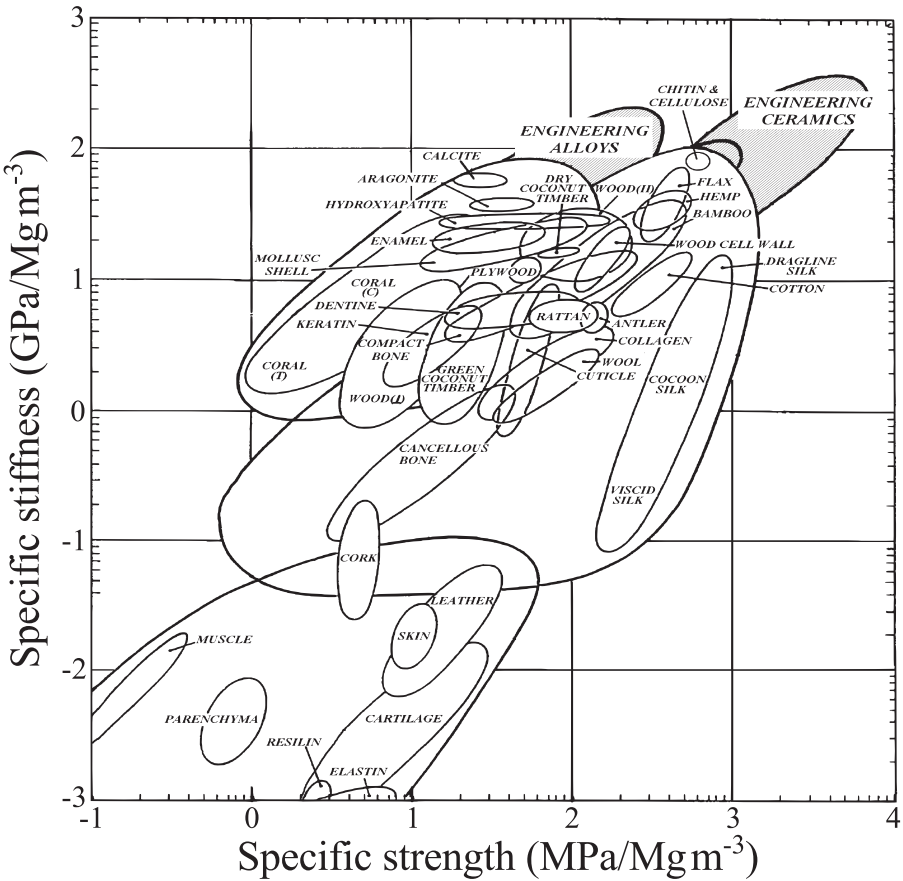


Figure 7.19. Material map showing engineering materials hidden by biological materials. Synthesized from papers by Ashby and Wegst.

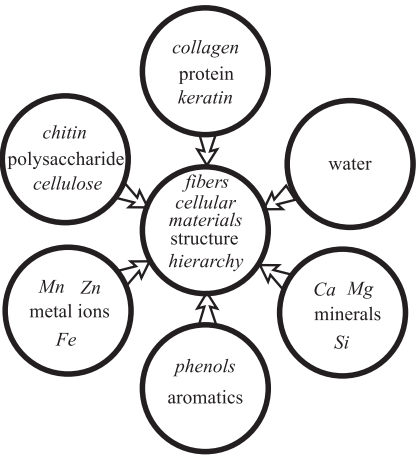


Figure 7.20. Contributions to biological materials shown in figure 7.19.

in which a fiber, itself a composite like the collagen–apatite fibers of bone, could be extruded in a rapid manufacturing machine and incorporated into a hierarchical composite with orientations in both fiber and matrix. Rapid manufacturing can already produce cellular structures rather like biological structures (Stampfl et al. 2004), but these have little or no hierarchy. The next phase of biomimetics should therefore be to look more closely at the processes that nature uses to produce morphology. Although these are studied in embryology and cellular morphogenesis (Green, Steele, and Renich 1996; Nelson et al. 2005; Savill and Hogeweg 1997), very little has been done to apply these observations to a nonliving technical environment (Thom 1975; Thompson 1959). Biomimetics can point the way toward a more sustainable future, but it is not sufficient to translate its lessons into the present technology. It is necessary to translate that technology into a biological format.

In terms of the Inventive Principles, “Information” is a bit of a disappointment. TRIZ has only four such Principles, which is a distinct underrepresentation but is an honest reflection of the paucity of information-based techniques in technology. It is very likely that the gap between biology and technology will be filled with information. All we have from TRIZ is “Feedback,” although, of course, this is one of the most powerful design principles for an autonomous system and is becoming prevalent with computer control of systems.

7.6.1 FEEDBACK

Feedback improves a process or action; modulated feedback brings further improvement. For instance, the level of fuel in a carburetor adjusts itself via a floating valve placed inside the temporary fuel tank. Quality of a product is best measured during production, rather than after it, since the process can be corrected immediately when a fault is noticed. In business, systems for getting feedback from customers are continually being improved. Feedback is a primary learning mechanism.

All organisms have built-in feedback, since the chemical reactions that maintain them can occur under only relatively controlled conditions. Feedback can be chemical (cellular metabolism), neural (fine-tuning optical or aural response), neuromuscular (coordinated movements), and so forth. Nature is replete with feedback mechanisms, since they can greatly improve the performance of a wide variety of functions that are not mechanically perfect or optimized. A particularly good example is the use of feedback in frequency analysis in the middle ear. There is also much feedback in the control of population density. Feedback is probably the most ignored asset of biomimetics.

7.7 . . . And Structure . . .

Among the Inventive Principles that come under the heading of “Structure” are “Local property” (identified as the most important principle in a design study of insect cuticle [Vincent 2005]), “Segmentation,” and “Mediator.” Taken in order, these suggest the following.

7.7.1 LOCAL PROPERTIES

At the biological level, this is probably where evolution belongs, since the basic expansion of an evolutionary series relies on the development of small adaptations within an overall structure or bauplan (figure 7.21). Each internal organ in an animal has its own microenvironment and is often surrounded by a membrane that emphasizes this separation. Every organism has to be multifunctional, even a single cell, and so is very heterogeneous. There is much to be learned here regarding the optimization of the various functions, spatial separations, and functional integrations involved. The internal organs of invertebrates tend to have more autonomy, which possibly makes the organs (and therefore the individuals) more robust.

At the technical level, change the structure of a system or object from uniform to nonuniform; change an external environment (or external influence) from uniform to nonuniform. Make each part of an object or system function in conditions most suitable for its operation. Make each part of an object fulfill a different and useful function (for example, a dust filter is made of porous membranes; the outer membrane has large pores; the inner one, small pores). Employ quenching and other treatments of the surface layer of metal components make the surface properties different from the bulk properties of the material. Use the appropriate sized spanner for every nut, because fixed-size spanners are much stronger than adjustable spanners.

Specialized compartments in a lunchbox for each type of food keep hot things hot and cold things cold, and make it safe and economical for workers and schoolchildren to carry their lunches with them. In business the segmentation of the market illustrates the local quality principle, too. Segmentation is used to divide the market into small markets with specific attributes, then local quality is used to treat each of those markets appropriately. The Whirlpool Company has hired marketing people who speak 18 different languages in India, to tailor its approach to the automatic washing machine market to the cultural preferences of each group. Local quality applies to people, too. Some are most effective working on their own, and others are

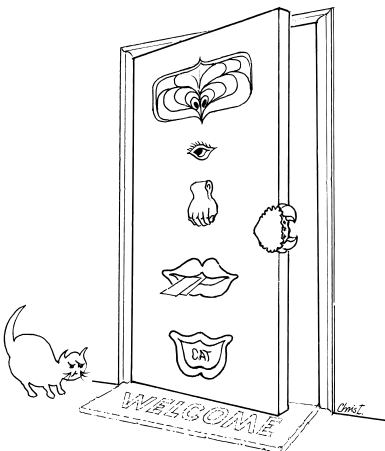


Figure 7.21. TRIZ Inventive Principle No. 3, “Local quality” (© C Isaac).

most effective in teams. Deep professional specialization is needed for certain skills, and a broad liberal arts background is needed for other situations.

7.7.2 SEGMENTATION

At the technical level, divide an object into independent parts, make an object easy to dismantle, or increase fragmentation (figure 7.22). Biologically, division of an “object” is seen in insect colonies when groups of ants, bees, or wasps act as a coherent unit to defend the nest or to swarm. Many animals are simply segmented (which probably makes them simpler to define at the genetic level, or, from an evolutionary point of view, the animal can become larger with very little addition to the genetic control), and most plants are modular (leaves, flowers, stem, root). Modern genetics shows that the underlying instructions for forming different parts of an organism are very similar, so that an insect’s antenna can be persuaded to develop as a leg. Ease of dismantling is seen in autotomy and abscission mechanisms in which parts of animals and plants can be made to fall off in a controlled manner. Examples are autumnal leaf fall and loss of limbs or tail by many animals when challenged. It is a general rule in structural design that compressive and tensile elements should be minimized and kept separate, and reducing either one (usually the compressive element) to zero will allow the least amount of the other element (tensile) to be used. This phenomenon is seen in animals and plants with hydrostatic skeletons (which accounts for all nonwoody plants) and can be done only with prestress (compare with earlier “Prior Counteraction”).

The following are examples from technology. Connect the elements of the pole of a temporary street light with flexible joints for easy transport and installation. Use blinds or miniblinds instead of continuous shades. Divide a large truck into a tractor and trailer. Use Java applets instead of a single large program.

The segmentation of the market is a common practice and a commonly used term. A corporation should be small, to be flexible, but at the same time it should be big, to have enough resources for production and marketing. To get small and big at the same time, a huge company is divided into subsidiaries, profit centers, or other business units that work relatively independently. For the last 30 years, the use of teams has been persistent in the workplace, because small teams are flexible and can make decisions quickly. A large job can be broken into many smaller jobs (called a “work breakdown structure” in project management). The JIT (just-in-time or Kanban) system uses the concept of segmentation to an extreme—it replaces the idea of mass production with the idea that the most efficient production system can produce a single unit just as easily as multiple units.

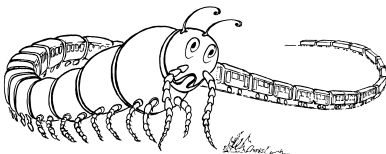


Figure 7.22. TRIZ Inventive Principle No. 1, “Segmentation” (© C Isaac).

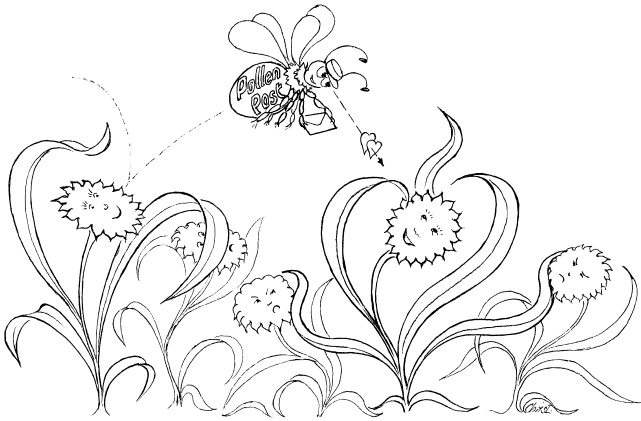


Figure 7.23. TRIZ Inventive Principle No. 34, “Mediation” (© C Isaac).

7.7.3 MEDIATOR

Biologically, think of parasitism, in which one animal or plant lives inside another for a greater or lesser part of its life cycle; the use of tools; pollination by insects (figure 7.23); an enzyme; any molecule that binds some other molecule, such as an adhesive or water-binding space-filler—all are examples of mediators or mediation.

At the technical level, an intermediary carrier or process is used to merge one object temporarily with another. To make a diamond-coated disk, the diamond powder is deposited onto a sacrificial layer that is applied to the disk then dissolved, leaving the diamond on the surface of the disc. Fixtures or jigs are used to position parts to make assembly easy. The assembled parts are removed, and the jigs are used over and over again. This concept is applied in the home as well as in the factory—a baking pan or a gelatin mold is an “intermediary” for shaping a dessert, and a potholder is an intermediary for carrying a hot dish to the table without burning the server’s hands. Ice can be used to hold small components in place temporarily if they will not be harmed by water when the ice melts. A neutral third party can be used as an intermediary during difficult negotiations. For sales promotion, an intermediary who is seen by the customer as an impartial expert can make recommendations.

7.7.4 . . . AND BACK IT UP WITH HIERARCHY

Hierarchy in biological materials and structures (figure 7.24) has been talked and written about for a long time but does not seem to have been approached in a particularly objective manner or had its advantages quantified until recently. Rod Lakes of the University of Wisconsin showed that not only is the Eiffel Tower a level 3 hierarchical structure (it’s made out of struts made from struts made from struts, as illustrated in figure 7.25) but it is 10 times more efficient in its use of material than the nearby Pompidou Center (Lakes 1993). He also showed experimentally that a

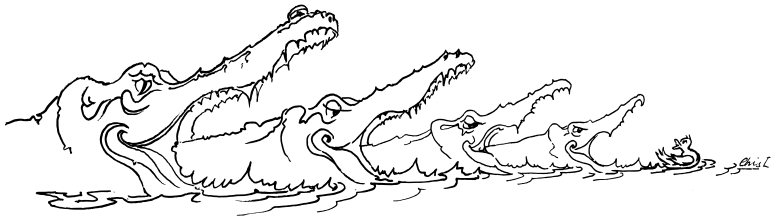


Figure 7.24. TRIZ Inventive Principle No. 7, “Nested doll” (© C Isaac).

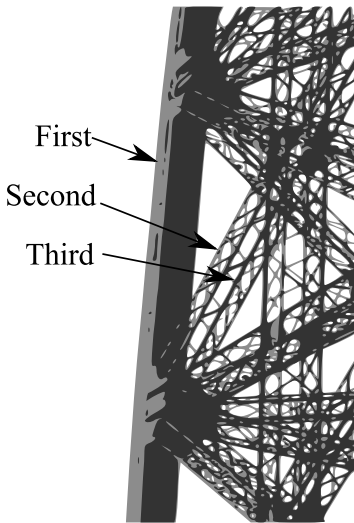


Figure 7.25. Part of the Eiffel Tower showing struts at three levels of hierarchy.

second-order honeycomb structure (i.e., a honeycomb in which there are some large holes) of the same weight per unit area as a first-order honeycomb (which is what is normally used, and what you see in a beehive whose cells—at least those used for storage and for rearing the worker larvae—are all the same size) is more than four times stronger in compression. The explanation is that the walls in the second-order honeycomb are thicker (and thus the weight per unit area is kept constant), and the resistance to buckling is a nonlinear function of the wall thickness.

The second-order honeycomb is a very appropriate model for the wood from an angiosperm tree, in which the large vessels (0.5 mm in diameter) that carry water up the trunk are 10 times larger than the surrounding xylem parenchyma cells. In three dimensions, the inner structures of leaves of emergent aquatic plants such as lilies and reed mace are second-order hierarchical structures. More recent work on the hierarchy of bone (as a model material) has concentrated on the transfer of shear from one level of hierarchy to the next, beginning with a crystal of hydroxyapatite, which is so small that it cannot contain a Griffith flaw and therefore cannot fail

catastrophically (Z. Zhang, Y. Zhang, and Gao 2011). Unfortunately, these models seem too “perfect,” and repeat identical structure at different sizes, which does not happen in biological organisms. Nor do the models allow for the realities of nonlinear effects such as those found in nacre (Barthelat et al. 2007) or the complexities of real hierarchical morphology, which is rarely if ever fractal, a commonly made but erroneous assumption.

7.8 Well, That's It

I've come to the end of my story, or stories. We are supposed to be entering the age of the life sciences, and that is very probably true. Biology is not the “stamp collecting” that Rutherford wanted us to believe. It's probably the most complex of the sciences. But many people confuse complexity and triviality: if they can't understand something, it's more comforting to say it's unimportant. In fact, large parts of biology are not science yet—they are still in the descriptive stage. Biomimetics is one of these.

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